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CHEMISTRY OF THE COTTON PLANT, WITH SPECIAL REFERENCE TO UPLAND COTTON[1]

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THE PROBLEM

The main purpose of the investigation reported in this paper was to isolate the substance which proves so attractive to the boll weevil, an attraction causing such disastrous losses to the cotton industry.[2] While this paper chiefly concerns the isolation of the glucosids and their products of hydrolysis, preliminary studies of an ethereal oil which has been isolated from different parts of the cotton plant are also discussed. This oil has been found decidedly attractive to the boll weevil.

It was deemed important, furthermore, to ascertain whether or not cotton (*Gossypium* spp.) grown in this country, and especially the Upland species (*Gossypium hirsutum*), contained any substances isolated previously by Perkin from Indian or Egyptian types. Since results of the writers brought out the fact that Upland cotton was different in chemical composition from any of those previously investigated by Perkin, it was considered advisable to report briefly his work on the chemistry of other types of cotton. These investigations, it is hoped, will emphasize the importance of establishing definitely the type of cotton used in any further work.

I.—THE GLUCOSIDS AND THEIR PRODUCTS OF HYDROLYSIS

During the course of his investigations Perkin (4)[3] found that the white flowers of Indian cotton (*G. neglectum* var. *roseum*)[4] were devoid of dyeing properties, and the pink flowers of *G. sanguineum* contained only traces of a coloring substance, probably quercetin. The flowers of Egyptian cotton, the yellow flowers of common Indian cotton (*G. herbaceum*), as well as the yellow flowers obtained from another Indian cotton (*G. neglectum*) contained isoquercitrin and gossypitrin. Usually quercetin and gossypetin could be found in the extracts as products of hydrolysis of these glucosids. No gossypitrin could be found in the red

[1] This paper is the first of a series on the chemistry of the cotton plant.
[2] This work was done in cooperation with the Bureau of Entomology.
[3] Reference is made by number (italic) to "Literature cited," pp. 351-352.
[4] Perkins gives "rossrum," an evident typographic error for "roseum."

cotton flowers of *G. arboreum* L., from which, however, isoquercitrin and quercetin were isolated. Quercimeritrin, another glucosid, could be isolated only from one of the types mentioned—namely, the Egyptian cotton.

As a result of these investigations, the writers found that the petals of Upland cotton and the flowers with petals removed contained appreciable amounts of quercimeritrin. Very small amounts of isoquercitrin could also be found in the petals. So far, no gossypitrin or gossypetin could be isolated. The leaves were collected from plants grown at Brownsville, Texas, and the flowers from plants at Tallulah, Louisiana.

The results showing the character and distribution of color substances in the different types of the cotton plant are compiled in Table I, which shows clearly the chemical distinction among the different types of the cotton plant, as recently indicated by Perkin.

TABLE I.—*Distribution of glucosids and their products of hydrolysis in cotton*

Common name.	Scientific name.	Part examined.	Glucosids.		Product of hydrolysis.	Glucosid.	Product of hydrolysis.
			Quercimeritrin.	Isoquercitrin.	Quercetin.	Gossypitrin.	Gossypetin.
Egyptian cotton	*Gossypium barbadense.*	Flowers	Present.	Present.	Present.	Present.	Present.
Upland cotton	*G. hirsutum*	Leaves	do		do		
Do	do	Flowers with petals removed.	do				
Do	do	Petals	do	Present.			
Common yellow Indian cotton.	*G. herbaceum*	Flowers		do	Present.	Present.	Present.
Yellow Indian cotton.	*G. neglectum*	do		do	do	do	Do.
Red cotton	*G. arboreum*	do		do	do		
Pink cotton	*G. sanguineum*	do			do		
White Indian cotton.	*G. neglectum* var. *roseum.*	do					

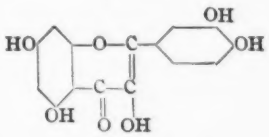
For the sake of comparison, the chief properties of the glucosids isolated from the cotton plant and their products of hydrolysis, compiled largely from the work of Perkin, are described below.

The glucosids quercimeritrin and isoquercitrin are isomeric compounds with the formula $C_{21}H_{20}O_{12}$. Isoquercitrin (*3*) has been found only in the cotton plant, while quercimeritrin (*1*) has also been found associated with prunitrin in the bark of a species of Prunus, closely related to *Prunus emarginata*.

QUERCIMERITRIN (*3*) crystallizes with three molecules of water from pyridin and water in small glistening plates of a bright-yellow color. They melt in a very pure state at 247 ° to 249° C., are almost insoluble in cold water or alcohol, but are rather easily soluble in the hot solvents. The acetyl derivative consists of needles that melt at 214° to 216° C. and are difficultly soluble in boiling alcohol.

ISOQUERCITRIN (*3*) crystallizes from pyridin and water in pale-yellow needles that melt at 217° to 219° C., are almost insoluble in cold water, sparingly soluble in boiling water. The acetyl derivative is much more soluble in alcohol than that of quercimeritrin. By hydrolysis both glucosids are split into quercetin and dextrose. Isoquercitrin is more easily hydrolyzed than is quercimeritrin.

QUERCETIN, a product of hydrolysis of quercimeritrin and isoquercitrin, is a flavone derivative to which the following formula has been assigned by various investigators (*10*):



Quercetin (*9*) has been found quite abundantly in various plants, either free or combined. Since some of these quercetin compounds are easily hydrolyzed, the finding of free quercetin may often be due to the occurrence of hydrolysis during the extraction. Most of the quercetin compounds are glucosids, the nature of the glucosid depending on the sugar groups with which quercetin is combined and also on the place of linkage. Among such glucosids not occurring in the cotton plant, quercitrin (*9*), rutin (*9*), and serotrin (*6*) have been isolated. The sugars obtained by the hydrolysis of these glucosids are dextrose and rhamnose.

Quercetin crystallizes from aqueous solutions with two molecules of water in fine needles of a bright-yellow color. It melts with partial decomposition at about 310° C.[1] It is only slightly soluble in boiling, and almost insoluble in cold water.

The solubility in boiling alcohol is 1 to 18, being more than 10 times the solubility in cold alcohol. The acetyl derivative crystallizes in colorless needles that melt at 194°.

GOSSYPITRIN[2] (*3*), another glucosid found in cotton, has the formula $C_{21}H_{20}O_{13}$. It crystallizes in small glistening needles of an orange-yrllow color and melts at 200-202°C. It is only slightly soluble in water and alcohol. The acetyl derivative crystallizes from acetic anhydrid and alcohol in colorless needles melting at 226° to 228° C. They are almost insoluble in alcohol.

[1] There is some confusion in the literature concerning the melting point of quercetin, some authors (*7 p. 524*) stating that it melts at 251° C., others (*8, p. 813*) at 310°. The writers found that their quercetin melted at 310°. The same was true of a commerical sample. Both of these samples of quercetin gave acetyl-quercetin melting at 194°.

[2] The presence of this glucosid in *Hibiscus sabdariffa* and *Thespasia lampas* has been reported by Schmidt, but the authors have been unable to locate the original references. (SCHMIDT, Ernst. AUSFÜHRLICHES LEHRBUCH DER PHARMAZEUTISCHEN CHEMIE. Aufl. 5, Bd. 2, p. 2040. Braunschweig, 1911.)

GOSSYPETIN.—By the hydrolysis of gossypitrin Perkin obtained gossypetin and a sugar identified as dextrose. Gossypetin has also been found as a glucosid in *Hibiscus sabdariffa* (*2*). It crystallizes in yellow needles melting at 311° to 313° C. They are slightly soluble in water, but are easily soluble in alcohol. Acetylgossypetin crystallizes from a mixture of alcohol and acetic acid in colorless needles melting at 228° to 230° C. These are readily soluble in acetic acid, but are somewhat sparingly soluble in alcohol.

EXPERIMENTAL WORK

ISOLATION OF QUERCIMERITRIN FROM THE PETALS

Quercimeritrin was isolated by Perkin's method (*3*), which was modified by omitting the use of lead acetate and was then employed in the following manner: An alcoholic extract from 1,214 gm. of air-dried, powdered petals was evaporated to a volume of about one and one-half liters. The deep purple-red solution was filtered from the dark material which had separated. This residue was warmed with water and filtered. On standing, an amorphous brick-red substance separated. This was filtered off by suction, boiled in absolute alcohol, and again filtered hot. On cooling, the yellow glucosid deposited in microcrystalline form, melting at 243° C. When mixed with known material obtained from petals by Perkin's original method, no lowering of the melting point was observed.

ISOLATION OF ISOQUERCITRIN FROM THE PETALS

In another experiment the alcoholic solution was evaporated to a small volume, and water was added. The mixture was then evaporated until most of the alcohol was gone. After extracting the aqueous solution with ether, neutral lead acetate was added. The heavy, dull-yellow precipitate produced by this reagent was filtered off. A thin paste was then made with water, and the lead removed by passing hydrogen sulphid through the mixture. After filtering, the filtrate was evaporated somewhat and allowed to stand in a vacuum desiccator for several days, when small greenish-yellow needles separated. On recrystallization from dilute methyl alcohol several times, the product finally melted at 247° C. The substance was undoubtedly quercimeritrin.

The filtrate from the lead acetate precipitation was treated with basic lead acetate, which produced another heavy dull-yellow precipitate. The lead was removed from this precipitate as in the previous case, and the solution was slowly evaporated over sulphuric acid in a vacuum desiccator. From this solution there was obtained by fractional crystallizations more quercimeritrin, and from the mother liquors a crystalline substance melting, after purification from pyridin and water, at 219° C. Hydrolysis of this compound yielded quercetin. It was consequently identified as isoquercitrin.

ISOLATION OF QUERCIMERITRIN FROM THE LEAVES

The leaves were treated according to Perkin's original method, as follows: Air-dried, pulverized cotton leaves (1,500 gms.) were heated with alcohol in an aluminum kettle for 4 hours on a steam bath. After cooling, the extract was pressed out and the dark-green liquid evaporated in a vacuum still to a small volume. Hot water was then added, and the distillation was continued until all the alcohol was removed. The hot mixture was then allowed to stand for about an hour, when an upper layer of black tar formed. The warm aqueous solution underneath was siphoned off and filtered through paper pulp. To remove chlorophyll and waxy matter, the cooled siphonate was twice extracted with ether, and the resulting clear, red, aqueous solution heated to expel any remaining ether. Lead-acetate solution was then added, and the thick orange-yellow precipitate that formed was filtered off by suction and washed with water. The lead was then removed from this precipitate by mixing it with hot water to make a thin paste, through which hydrogen sulphid was passed. The lead sulphid was filtered off by means of paper pulp. The clear, red filtrate was evaporated to a small volume and allowed to stand for several days, when a yellow amorphous substance slowly separated. Several fractions of this material were further obtained from the mother liquor. These fractions consisted of much quercetin and a little quercimeritrin. By a series of recrystallizations from water dilute alcohol, dilute acetic acid, and from pyridin, the quercimeritrin was obtained in a comparatively pure state. The quercimeritrin thus obtained melted at 247° C. When hydrolyzed with 5 per cent sulphuric acid, it gave quercetin. The latter compound was identified by the fact that it gave an acetyl derivative melting at 194° C. A mixture of quercimeritrin from the petals and from the leaves also melted at 247° C.

ISOLATION OF QUERCIMERITRIN FROM THE FLOWERS WITH PETALS REMOVED

These parts of the plant, treated in the same manner as the leaves, also gave quercimeritrin melting at 247° C.

II.—THE ETHEREAL OIL

While a volatile oil had previously been isolated in small amounts from the bark of the root of *Gossypium herbaceum* by Power and Browning (5), none had been reported from aboveground parts of this plant nor of other species of Gossypium.

The first intimation that a volatile oil might be present in plants of the Upland cotton was obtained by observing that a steam distillate of the leaves proved to be attractive to the boll weevil. The amount of oil found in the leaves or young plants was very small. In over 4,000 pounds of fresh plants only about an ounce of volatile oil was secured, amounting to 0.0015 per cent on an average.

The highest results from plants so far examined were yielded by squaring plants—namely, 0.0054 per cent in one and 0.0071 in another case. However, not sufficient data are on hand to allow any definite conclusions as to the relative yield from plants in different stages of growth.

The yield from the Sea Island cotton was about the same as that found in Upland cotton. The oil was of a more or less brown color, distilling over mainly between 200° and 300° C. at atmospheric pressure, the lower fractions having a yellow, the higher a blue color. The volatile oil obtained from cotton bark by Power and Browning had a pale-yellow color and distilled between 120° and 135° under the same conditions.

The oil isolated by the writers did not give any furfurol reaction nor deposit any crystals, and was also in these respects different from the oil isolated by Power and Browning, which gave a furfurol reaction and deposited crystals that crystallized from ethylacetate as needles melting at 112° to 114°, and consisted apparently of acetovanillon. Table II is of interest, since it shows the character and amount of plants used and the amount of oil yielded.

TABLE II.—*Results of distillations of cotton plants*

Date.	Weight of plants.	Oil yield. Weight.	Percentage of plant weight.	Description of plants.
	Pounds.	*Gm.*		
May 2 and 3	1,444	2.933	0.0004	Seedlings.
May 4	24	.586	.0054	Squaring plants from hotbed.
May 5	530	4.253	.0017	Seedlings.
May 8	86	.765	.0017	Do.
Do	87	.240	.0006	Do.
May 9	160	1.347	.0018	Do.
Do	124	1.194	.0021	Do.
May 21	25	.409	.0036	Do.
May 26	55	.380	.0015	Do.
May 31	431	7.536	.0038	Do.
June 1	4	.130	.0071	Squaring from hotbed.
June 4 and 5	528	3.760	.0016	Sea Island cotton.
June 6 and 11	624	5.400	.0019	Field cotton squaring.
Total of lots	4,122	28.933	.....	
Average of lots	.....	.....	.0015	

EXPERIMENTAL WORK [1]

The fresh plants, collected in the vicinity of Tallulah, La., were distilled as soon as possible with steam in a specially constructed apparatus. The distillate was shaken out with ether and the ether removed by placing the container in warm water, finally using water of about 50° to 60° C. All ether was considered removed when the oil showed little or no loss in weight after a few minutes' heating at this temperature.

[1] The kind assistance of Mr. L. A. Sallinger, of the Savannah Laboratory, and Dr. A. R. Albright, of the Food Investigation Laboratory, is hereby gratefully acknowledged.

In the experiment concerned with the fractional distillation, 7.84 gm. were heated on the steam bath for two hours. At the end of that time the oil had apparently lost weight to the extent of 0.04 gm., the loss consisting of ether. The heated sample was then fractionated at ordinary pressure, giving the following results.

Fraction.	Quantity of distillate.	Temperature of distillate.	Color.
		°C.	
1	4 to 6 drops	Up to 200	Light-yellow.
2	About 1 c. c.	200–250	Greenish yellow.
3	About 1 to 2 c. c.	250–270	Light blue-green.
4	2 to 3 c. c.	270–285	Dark blue-green.
5	Less than 1 c. c.	285–300	Dark blue.
6	Few drops	300–325	Darker blue
Residue	About 1 c. c.		Black.

The odor of the residue only seemed empyreumatic.

SUMMARY

(1) Quercimeritrin and isoquercitrin, formerly isolated from other types of the cotton plant, have now also been found in Upland cotton (*Gossypium hirsutum*).

(2) The leaves and flowers, with petals removed, contained quercimeritrin, while the petals contained both quercimeritrin and isoquercitrin.

(3) No traces have been found of gossypitrin and gossypetin, which have been isolated from other types of cotton.

(4) An ethereal oil has been isolated from *G. hirsutum* which is different from that found in the bark of the root of *G. herbaceum*. It distills mainly between 200° and 300° C., and leaves a black empyreumatic residue. The lower fractions of the distillate have a yellow to greenish-yellow color, the higher fractions light blue-green to dark blue. This oil proved to be attractive to the boll weevil.

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STABILITY OF OLIVE OIL[1]

By E. B. HOLLAND, *Associate Chemist*, and J. C. REED and J. P. BUCKLEY, Jr., *Assistant Chemists*,[2] *Massachusetts Agricultural Experiment Station*

INTRODUCTION

Some years ago one of the writers reported the results of an experiment to determine the effect (*4*)[3] of air, light, and moisture at room temperature on butter fat. The test was planned to show the action of the three agents, singly and in combination, and was continued for a year and a half. The experiment furnished considerable information relative to the changes that take place in such materials, but proved faulty in that a fat, solid and opaque at ordinary temperature, was a poor medium for measuring such changes, which evidently were not uniform throughout the mass, but greatest at the surface; furthermore, the conditions surrounding the fat were not under satisfactory control.

Conceding the limitations of the previous experiment, but recognizing the economic value as well as scientific interest of such investigations, the writers deemed it advisable to conduct another series of tests, under more definite conditions. For this purpose all oils procurable in quantity at a reasonable price were carefully considered. Olive oil was finally selected for the reason that it is a well-known edible product of fair keeping properties and of the composition desired.

THE OIL EMPLOYED

A few letters of inquiry to Federal and Experiment Station officials elicited the information that pure olive oil, both foreign and domestic, was readily obtainable. As an American oil could be procured directly from the pressers in a comparatively short time, with details of production and treatment, an order was placed, specifying an absolutely pure product that had not been bleached, sterilized, or refined in any way except as to filtration and having a low content of free fatty acids.

A 5-gallon can of California olive oil was received on February 25, 1910. The manufacturer stated that the oil was cold-pressed from an average run of hand-picked, washed, and ground ripe California-grown olives of the season of 1908. After extraction, the oil was pumped into settling tanks and from there to storage tanks, whence it was filtered four to six times through French filter paper in a special press and was not put on the market until it was at least a year old.

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[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Mr. Reed was associated with the senior writer in the earlier stages of the work and Mr. Buckley in the later.

[3] Reference is made by number (italic) to "Literature cited," p. 366.

ORGANOLEPTIC TESTS

The appearance of the oil as determined by the unaided eye varied with the depth of stratum and character of the light from transparent olive-green to opaque, almost black. All efforts at color differentiation without an instrument proved unsatisfactory, but were continued throughout the experiment, as a tintometer was not available for the first four years. In amounts of 6 ounces, the basis employed, the oil will be designated a dark olive-green. The green greatly exceeded that in most olive oils offered in local markets, probably due, as the manufacturer claims, to differences in soil and climatic conditions, together with possibly small variations in manufacturing methods. The oil had an excellent body and a pronounced olive odor.

PHYSICAL TESTS

Specific gravity $\frac{20^\circ}{20^\circ}$ C.	0.91308
Specific gravity $\frac{20^\circ}{4^\circ}$ C. (calculated)	0.91152
Specific gravity 25° C. (U. S. P. standard)	0.910–0.915
Refractive index $n \frac{20^\circ \text{ C.}}{D}$ (Abbe)	1.4687
Viscosity 70° F. (Redwood)	12.0
Valenta test (B. and A. 99.5 per cent acid)	87.5° C.
Elaidin test	Green, semisolid

CHEMICAL TESTS

Saponification number	190.636
Saponification number (U. S. P. standard)	190–195
Acid number (a)	1.990
Ether number (e)	188.646
Total fatty acids (1.00–0.00022594e)	95.74 per cent
Neutralization number (n)	199.12
Mean molecular weight	281.78
Free fatty acids as oleic acid and as $\frac{a}{n}$	1.00 per cent
Glycerol (0.00054703e)	10.32 per cent
Reichert-Meissl number	None
Polenske number	0.13
Insoluble acids	95.40 per cent
Iodin number (Wijs)	83.45
Iodin number (U. S. P. standard)	79–90
Acetyl number	5.69

COLOR TESTS

Baudouin test for sesame oil	Nil
Bechi silver nitrate test for cottonseed oil	Nil
Halphen test for cottonseed oil	Nil
Nitric-acid test for seed oils	Brown, slight coagulation

All organoleptic, physical, chemical, and color tests indicated a pure olive oil, with the exception of the nitric-acid test which may be disregarded, as it is no longer designated by the Pharmacopœia.

PLAN OF THE EXPERIMENT

The object of the investigation primarily was to ascertain the nature and extent of the action of the several agents upon the oil as determined by changes in physical characteristics and chemical composition; and secondarily to deduce, if possible, from the results obtained a practical method for handling commercial oils. The experiment was planned to demonstrate the effect of air, light, and moisture, singly and in combination, which, together with control (the basis for comparison) and enzym-free samples, required nine series of tests, as follows:

Series.	Condition of the experiment.
A	Control.
B	Enzym-free.
C	Air.
D	Light.
E	Moisture.
F	Air-light.
G	Air-moisture.
H	Light-moisture.
I	Air-light-moisture.

As the change in the oil in most cases would be comparatively slow, six years were believed necessary to obtain the maximum effect desired. The previous experiment having demonstrated that analysis oftener than once a year did not compensate for the extra labor involved, only 1 sample was alloted for each year, or 6 for each series, making a total of 54 samples. Six ounces of oil were taken for each sample which was insufficient for some physical tests, but ample for most chemical. Round flint-glass bottles of 6-ounce capacity with glass stoppers were used as containers after being carefully cleaned and dried.

Five c. c. of distilled water were pipetted into each bottle of series E, G, H, and I, after which all the bottles, with the exception of series B, were filled with the oil as received, after it was thoroughly mixed to insure uniformity. Another portion of the oil, used for series B, was heated to 70° C. on two successive days for approximately 60 minutes on the first day and 30 on the second, to destroy enzyms, if any were present. All bottles were filled to the shoulder. Where air was not a factor, the bottles were closed with glass stoppers and carefully sealed with wax. Such treatment failed as a control measure, as a small amount of air remained in the bottles; but this appeared unavoidable under the circumstances. Each series of tests was inclosed in an 8-inch Fruehling and Schultz desiccator after the porcelain plate had been removed. The bottles in an upright position were arranged in a circle and well spaced.

To exclude the action of moisture (series A, B, C, D, and F) sulphuric acid, previously heated in most cases to 212° C., or higher, was poured into the desiccators to absorb any water that might gain access. To secure a saturated atmosphere (series E, G, H, and I), distilled water was poured into the desiccators, in addition to the water in the bottles.

To exclude air (series A, B, D, E, and H), the desiccators were rarefied by means of a vacuum pump, and a small U-shaped manometer was suspended from the hook of the stopcock to indicate the rarefaction and its

permanence. The joints of these desiccators were covered with wax; but even under the best conditions leakage could not be prevented entirely, and it was found necessary to pump out the desiccators several times a year. To obtain the effect of air (series C, F, G, and I) the glass stopcocks of the desiccators were replaced by perforated rubber stoppers and straight glass tubes which passed through the stoppers and dipped into the sulphuric acid (series C and F) or into the water (series G and I).

To exclude light (series A, B, C, E, and G), the desiccators were placed in a large oblong wooden box, lined with building paper, with an overhanging cover similarly lined. The cover was held by corner posts 0.5 inch above the top of the box, projected 0.5 inch beyond the sides of the box, and overlapped 3.5 inches. In addition a strip 1.4 inches wide was nailed to the outside of the box 0.5 inch below the edge of the cover.

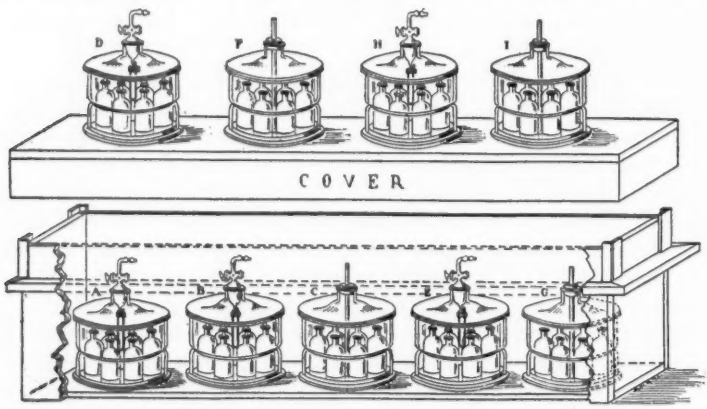

FIG. 1—Apparatus used in the experiments to determine the stability of olive oil.

This provided a continuous air passage 0.5 inch wide under all sides of the cover and yet absolutely prevented the entrance of light, even by reflection. To obtain the effect of light (series D, F, H, and I) the desiccators were placed on the cover of the box and exposed to light from a north window. All the samples were kept in the northeast room of the Experiment Station dairy building, into which the direct rays of the sun did not enter at any season of the year. The temperature was not constant, but relative in all cases. Considerable time was consumed in obtaining the necessary supplies and in preparing the samples, so that the experiment did not actually begin until April 2, 1910.

EFFECT OF AIR, LIGHT, AND MOISTURE

ORGANOLEPTIC CHANGES

Changes of an organoleptic character are difficult to measure and even more difficult to express, particularly where the differences are slight. The results are relative, however, if not strictly accurate, and are recorded in Table I.

TABLE I.—*Organoleptic changes in the olive oil*

Series.	Conditions of the experiment.	As received (1910).	After 1 year (1911).	After 2 years (1912).	After 3 years (1913).	After 4 years (1914).	After 5 years (1915).	After 6 years (1916).
A	Control	Clear; dark olive green; marked olive odor.	Clear; dark olive green; olive odor.	Clear; dark olive green; color and olive odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.
B	Enzym-free	do	do	do	do	do	do	Do.
C	Air	do	do	do	Less color than A; less odor than A.	Less green than A; slightly rancid odor (?).	Less green than A	Yellowish; rancid odor.
D	Light	do	Less green than A.	Less green than A	Greenish yellow	Greenish yellow	Slightly greenish yellow.	Slightly greenish yellow; slightly rancid odor.
E	Moisture	do	Turbid with small amount of precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with more precipitate than G, after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.
F	Air-light	do	Less green than D.	Light yellow, color largely destroyed; rancid odor.	Yellowish, nearly colorless; rancid odor.	Yellowish, nearly colorless; rancid odor.	Nearly colorless; rancid odor.	Nearly colorless; rancid odor.
G	Air-moisture	do	Clear with slight amount of precipitate; after filtering, color like A.	Clear with some precipitate, like E; after filtering, color like A.	Clear with least precipitate; after filtering, color like A; water acid to litmus; odor like C.	Clear with some precipitate, like E; after filtering, less green than C.	Slightly turbid with least precipitate; color less than D; rancid odor.	Slightly turbid with less precipitate than E; after filtering, slight color; rancid odor.
H	Light-moisture	do	Turbid with more precipitate than E; after filtering, color between D and F.	Turbid with more precipitate than G; after filtering, color lighter than D.	Turbid with more precipitate than E; after filtering, color like D; slightly rancid odor (?).	Turbid with considerable precipitate; after filtering, color between D and F.	Turbid with more precipitate than E; after filtering, more yellow than D.	Turbid with more precipitate than E; after filtering, color like D.
I	Air-light-moisture	do	Turbid with more precipitate than H; after filtering, color between H and F.	Turbid with most precipitate; after filtering, color like F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless like F; water acid to litmus; rancid odor.	Turbid with most precipitate; after filtering, less color than F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.

The color and characteristic odor of the oil seemed to diminish gradually in the control samples. The heated samples, series B, duplicated the control samples, so far as could be observed.

Air was a negligible factor for two years; then it effected a slow but marked destruction of color fully equal to light at the close of the experiment, and caused a rancid odor on the sixth year.

Light was active in destroying color and caused a slightly rancid odor on the sixth year, probably due to a small amount of inclosed air.

Moisture caused the formation of a precipitate which rendered the oil turbid, but which effected no apparent change in color after the removal of the precipitate.

Air-light was most active and effective in destroying color, equal to air-light-moisture, and produced a rancid odor on the second year.

Air-moisture caused the formation of a slight amount of precipitate but without appreciable turbidity until the fifth year, at which time a rancid odor was produced. Air-moisture was inactive as regards color for three years, but eventually exceeded the effect of air and equaled that of light.

Light-moisture affected the color about the same as light, and caused the formation of a considerable amount of precipitate which rendered the oil turbid.

Air-light-moisture affected the color the same as air-light, produced a rancid odor the second year, and caused the formation of probably the largest amount of precipitate, which rendered the oil turbid.

The chromogenic bodies of the oil were not appreciably affected by moisture, were destroyed slowly but effectively by air, slowly but rather more effectively by air-moisture, more actively by light and light-moisture, and most actively and effectively by air-light and by air-light-moisture. Air was slowly active, light probably assisted by a small amount of inclosed air more active, and air-light the most active in destroying color. Moisture was a negligible factor except possibly in the case of air-moisture.

A rancid odor was produced on the sixth year by air and by light, on the fifth year by air-moisture, and on the second year by air-light and by air-light-moisture. Neither air nor light alone was particularly active in producing rancidity, but jointly were decidedly effective. In this connection moisture did not appear to be a factor of any consequence.

In every instance the presence of moisture caused the formation of a precipitate in a relatively slight amount by air-moisture, in small amount by moisture, in a greater amount by light-moisture, and in apparently the largest amount by air-light-moisture. Light seemingly was a factor. The so-called precipitate was first observed as dirty-white or brownish-white spots on the sides of the bottle below the surface of the oil and might be said to resemble mold. As the amount increased, the bulk of it collected near the surface of the water layer or in the water. In no case was sufficient purified material obtained to make a chemical examination.

PHYSICAL TESTS

The refractive index of the different series was determined for a number of years by means of an Abbe refractometer. The readings did not indicate any appreciable change in the oil except with air-light and with air-light-moisture, where gains of approximately 0.001 were noted. The results for the year 1912 corrected are given in Table II. Those for 1913 and 1914 gave like differences and are not reported.

TABLE II.—*Refractive index for the olive oil, 1912*

Series.	Conditions of the experiment.	$n \frac{20^\circ C.}{D}$
A	Control	1.4687
B	Enzym-free	1.4687
C	Air	1.4690
D	Light	1.4690
E	Moisture	1.4690
F	Air-light	1.4700
G	Air-moisture	1.4689
H	Light-moisture	1.4689
I	Air-light-moisture	1.4702

A Lovibond tintometer was employed for determining the color of the oil in 1915 and 1916. The supply of standard glasses in 1915 was inadequate for satisfactory readings, particularly for the darker oils, and the results are merely indicative.

TABLE III.—*Color of the olive oil*

Series.	Conditions of the experiment.	Stratum.	Matching standards.			Color developed.	
			Red.	Yellow.	Blue.	Orange.	Yellow.
	September, 1915.						
		Inches.					
A	Control	0.50	1.0	6.8	.....	1.0	5.8
B	Enzym-free	.50	1.0	11.0	.....	1.0	10.0
C	Air	1.00	.5	1.4	.....	.5	.9
D	Light	.50	1.0	3.5	.....	1.0	2.5
E	Moisture	.50	1.0	7.3	.....	1.0	6.3
F	Air-light	1.00	.3	.8	.....	.3	.5
G	Air-moisture	1.00	.5	1.3	.....	.5	.8
H	Light-moisture	.50	1.0	3.5	.....	1.0	2.5
I	Air-light-moisture	1.00	.1	.7	.....	.1	.6
	March, 1916.						
A	Control	.25	1.0	12.8	.....	1.0	11.8
B	Enzym-free	.25	1.0	12.8	.....	1.0	11.8
C	Air	1.00	.7	1.8	.....	.7	1.1
D	Light	.50	.8	3.5	.....	.8	2.7
E	Moisture	.25	1.0	12.8	.....	1.0	11.8
F	Air-light	2.00	.5	1.5	.....	.5	1.0
G	Air-moisture	1.00	.2	1.8	.....	.2	1.6
H	Light-moisture	.50	1.2	4.6	.....	1.2	3.4
I	Air-light-moisture	2.00	.5	1.5	.....	.5	1.0

According to the tintometer readings, for the last two years (1915–16), the control, enzym-free, and moisture samples retained the most color; light and light-moisture next; air and air-moisture less; and air-light and air-light-moisture the least color. The organoleptic tests for the entire period rated light, and light-moisture more active than air and air-moisture, but equally effective at the close of the experiment, or nearly so. Differences between organoleptic and tintometer readings are due, partly at least, to the fact that the unaided eye is less sensitive to the yellow than to the darker colors and is unable to differentiate accurately between faint colors, but more particularly to failure in properly coordinating activeness or speed of destruction and effectiveness or completeness of destruction.

The viscosity of a "fractional" quantity of several of the samples was determined in 1912 by means of a Redwood viscosimeter.

TABLE IV.—*Viscosity of the olive oil, 1912*

Series.	Conditions of the experiment.	Viscosity.
A	Control	15.8
F	Air-light	20.5
I	Air-light-moisture	21.7

Air-light and air-light-moisture evidently increased the viscosity to a slight extent.

CHEMICAL TESTS

The decomposition of the olive oil as affected by air, light, and moisture, singly and in combination, was measured in terms of acid, saponification, and iodin numbers. At the outset the oil seemed to possess a certain resistance to hydrolysis, oxidation, etc., but after it began to break down to any extent, the changes were more rapid. The hydrolytic effect of air, light, and moisture on the glycerids of the oil was measured in terms of acid number, which indicates the amount of free fatty acids produced (Table V).

TABLE V.—*Acid number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	1.99	2.18	+0.19	2.29	+0.30	2.47	+0.48	2.66	+0.67	2.97	+.98	3.00	+1.01
B	Enzym-free		2.18	+0.19	2.20	+0.21	2.28	+0.29	2.39	+0.40	2.68	+.69	2.64	+.65
C	Air		2.07	+0.08	2.01	+0.02	2.03	+0.04	2.25	+0.26	2.60	+.61	2.96	+.97
D	Light		2.26	+0.27	2.38	+0.39	2.54	+0.55	2.86	+0.87	3.06	+1.07	2.39	+.40
E	Moisture		2.33	+0.34	2.55	+0.56	2.93	+0.94	3.48	+1.49	4.15	+2.16	4.59	+2.60
F	Air-light		2.10	+0.11	2.28	+0.29	2.78	+0.79	3.58	+1.59	4.94	+2.95	6.83	+4.84
G	Air-moisture		2.35	+0.36	2.60	+0.61	2.97	+0.98	3.69	+1.70	4.60	+2.61	5.67	+3.68
H	Light-moisture		2.46	+0.47	2.81	+0.82	3.29	+1.30	4.01	+2.02	4.86	+2.87	5.59	+3.60
I	Air-light-moisture		2.42	+0.43	3.09	+1.10	4.08	+2.09	5.88	+3.89	8.09	+6.10	12.45	+10.46

The control samples (the basis for comparison) hydrolyzed a little more than the enzym-free, although the differences were slight.

Neither air nor light showed any appreciable action.

Moisture was moderately active and gradually effected a noticeable amount of hydrolysis.

Air-moisture and light-moisture were rather more effective than moisture, although the influence of the air or of the light must have been secondary.

Air-light was inactive for two years; then it began to affect hydrolysis and eventually exceeded air-moisture and light-moisture, probably due to the impossibility of entirely excluding moisture under the conditions of operation.

Air-light-moisture was the first to effect an appreciable amount of hydrolysis and greatly exceeded all others at the close.

Moisture effected considerable hydrolysis; air-moisture and light-moisture caused an additional amount; air-light, probably assisted by some moisture, still more, and air-light-moisture was the most active and effective. Moisture was the essential factor although air and light together greatly accelerated it.

The decomposition of unsaturated acids of olive oil as effected by air, light, and moisture may be measured in a degree by the increase in the saponification number which indicates the amount of fatty acid of high molecular weight converted into acids of lower molecular weight (Table VI).

TABLE VI.—*Saponification number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	190.64	189.86	−0.78	189.25	−1.39	190.53	−0.11	190.62	−0.02	190.57	−0.07	190.07	−0.57
B	Enzym-free	.....	190.18	−0.46	189.98	−0.66	190.62	−0.02	190.79	+0.15	190.80	+0.16	190.60	−0.04
C	Air	.....	190.09	−0.55	190.26	−0.38	190.73	+0.09	192.24	+1.60	194.01	+3.37	195.36	+4.72
D	Light	.....	189.89	−0.75	189.58	−1.06	189.94	−0.70	189.88	−0.76	190.37	−0.27	190.45	−0.19
E	Moisture	.....	189.87	−0.77	189.18	−1.46	190.12	−0.52	190.03	−0.61	190.68	+0.04	189.80	−0.84
F	Air-light	.....	190.58	−0.06	191.79	+1.15	194.55	+3.91	195.10	+4.46	201.74	+11.10	203.68	+13.04
G	Air-moisture	.....	189.92	−0.72	190.19	−0.45	190.93	+0.29	191.84	+1.20	194.40	+3.76	194.77	+4.13
H	Light-moisture	.....	189.81	−0.83	189.89	−0.75	189.91	−0.73	189.64	−1.00	189.41	−1.23	189.82	−0.82
I	Air-light moisture	.....	190.44	−0.20	192.28	+1.64	194.07	+3.43	196.16	+5.52	200.16	+9.52	203.85	+13.21

The control, enzym-free, light, moisture, and light-moisture samples were not affected in total alkali-consuming power. Air and air-moisture caused a like increase in saponification number.

Air-light and air-light-moisture effected a greater increase and of like amount. Air was undoubtedly the principal factor, although greatly intensified by light.

Fittig (*1-3*) has shown that unsaturated acids with the double bond in $\beta\gamma$, or 9–10 position, as in oleic acid, may undergo intramolecular changes on boiling with caustic alkali, forming an α-β, or 2-3 acid, which on fusion with alkali, according to Varrentrapp (*9, p. 209–215*) and Molinari (*6, p. 293*), splits into acetic and another saturated acid.

$$\underset{\text{9-10 oleic acid}}{CH_3.(CH_2)_7.CH : CH.(CH_2)_7.COOH} = \underset{\alpha\text{-}\beta \text{ or 2-3 oleic acid.}}{CH_3.(CH_2)_{14}.CH : CH.COOH}$$

$$CH_3.(CH_2)_{14}.CH : CH.COOH + 2\ KOH = \underset{\text{palmitate}}{CH_3.(CH_2)_{14}.COOK} + \underset{\text{acetate.}}{CH_3COOK} + H_2$$

Schrauth (*7*) confirmed the reaction and claimed further that in general for each double bond two carbon atoms are split off in the form of acetic acid. Different authorities (*5, p. 353–354*) have shown that the unsaturated (liquid) acids of olive oil consist principally of oleic acid with a smaller amount of linolic acid.

After considering the high content of unsaturated acids and their possible decomposition as described, one might suggest as a tentative hypothesis that the increase in saponification number was due to the formation of a α-β, or 2-3 acid, from the action of air or more effectively from air-light, which on boiling with alcoholic potash broke down into acetic and palmitic acids. Either oleic or linolic acid might be affected, although the latter is unquestionably less stable and therefore more likely to undergo molecular change. Both oleic and linolic acids, on breaking down into acetic and palmitic acids, double their alkali-consuming power; therefore, the increase in saponification number would be directly proportional to the amount of acid affected. The statement made by Schrauth that one molecule of acetic acid splits off for each double bond, appeared untenable for linolic acid in this connection. The neutralization number of oleic acid is 198.709 and of linolic acid 200.138. Assuming that α-β, or 2-3 oleic acid and linolic acid, after molecular rearrangement have no appreciable iodin numbers, as determined by Wijs solution, the writers obtained the following results for the sixth year of the experiment (Table VII):

TABLE VII.—*Character of the decomposition of the olive oil (1916)*

Series.	Conditions of the experiment.	Increase in saponification number.	Equivalent to oleic acid.	Equivalent loss in iodin number.	Equivalent to linolic acid.	Equivalent loss in iodin number.	Actual loss in iodin number.[1]
			Per cent.		*Per cent.*		
C	Air	4.72	2.375	2.135	2.358	4.270	3.85
F	Air-light	13.04	6.562	5.899	6.516	11.800	11.88
G	Air-moisture	4.13	2.078	1.868	2.064	3.738	3.99
I	Air-light-moisture	13.21	6.648	5.976	6.600	11.952	11.68

[1] See Table VIII.

The close agreement of the calculated and the actual loss of iodin numbers on the basis of linolic acid would indicate that probably only linolic acid had been affected during the term of the experiment. The matter will be considered further under iodin number (Table VIII).

The decomposing action of air, light, and moisture on the unsaturated acids of olive oil may also be measured by the loss in iodin number (Table VIII.

TABLE VIII.—*Iodin number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	83.45	83.97	+0.52	83.05	−0.40	82.99	−0.46	83.85	+0.40	83.86	+0.41	83.88	+0.43
B	Enzym-free		83.84	+0.39	82.87	−0.58	83.09	−0.36	83.57	+0.12	83.97	+0.52	83.73	+0.28
C	Air		83.79	+0.34	82.52	−0.93	82.58	−0.87	81.97	−1.48	81.17	−2.28	79.60	−3.85
D	Light		83.97	+0.52	82.84	−0.61	83.01	−0.44	83.88	+0.43	83.99	+0.54	83.67	+0.22
E	Moisture		84.00	+0.55	83.17	−0.28	83.32	−0.13	83.80	+0.35	83.81	+0.36	83.79	+0.34
F	Air-light		83.23	−0.22	80.80	−2.65	79.49	−3.96	77.27	−6.28	75.31	−8.14	71.57	−11.88
G	Air-moisture		83.91	+0.46	82.79	−0.66	82.66	−0.79	82.43	−1.02	81.17	−2.28	79.46	−3.99
H	Light-moisture		83.97	+0.52	83.15	−0.30	82.95	−0.50	84.17	+0.72	83.99	+0.54	84.00	+0.55
I	Air-light-moisture		83.29	−0.16	80.84	−2.61	79.80	−3.65	77.29	−6.16	75.20	−8.25	71.77	−11.68

The control, enzym-free, light, moisture, and light-moisture samples were not affected. Air and air-moisture caused considerable loss and of substantially the same amount. Air-light and air-light-moisture were equally effective and caused much greater loss than air or air-moisture; presumably moisture was a negligible factor in both instances. Air was the principal factor, although greatly intensified by light. The loss in iodin number was proportional to the gain in saponification number evidently two different measurements of the same decomposition, as shown by Table IX. The iodin number of linolic acid is 181.091.

TABLE IX.—*Character of the decomposition of the olive oil (1916)*

Series.	Conditions of the experiment.	Loss in iodin number.	Equivalent to linolic acid.	Equivalent gain in saponification number.	Actual gain in saponification number.[1]
			Per cent.		
C	Air	3.85	2.126	4.254	4.72
F	Air-light	11.88	6.560	13.129	13.04
G	Air-moisture	3.99	2.203	4.409	4.13
I	Air-light-moisture	11.68	6.450	12.909	13.21

[1] See Table VI.

The formation of aldehyde as a result of oxidation was manifested by the color imparted to the alcoholic potash in the determination of saponification number (Table X). The fuchsin-aldehyde reagent (7, *p. 15*) was employed as a confirmatory test, but proved rather too sensitive for the purpose.

TABLE X.—*Production of aldehyde in the olive oil*

Series.	Conditions of the experiment.	As received, 1910.	1911	1912	1913	1914	1915	1916
C	Air				Trace	Present	Present	Present.
F	Air-light			Trace	Present	Considerable.	Considerable.	Considerable.
G	Air-moisture				Trace	Present	Present, like C.	Present, less than C.
I	Air-light-moisture.			Trace; more than F.	Present	Considerable.	Considerable; less than F.	Considerable; more than F.

The relative amount of aldehyde present in the samples was estimated by the depth of color produced. Air and air-moisture produced a small amount of aldehyde, which was first noticed on the third year. Air-light and air-light-moisture produced a larger amount, first noticed on the second year. Evidently air was the essential factor accelerated by light.

The original olive oil was carefully examined for enzyms by Dr. G. H. Chapman, of this Station, and although their presence was not detected, it is impossible to say whether any of the changes noted were induced or accelerated by their action.

DISCUSSION OF RESULTS

Air effected no appreciable change in the color of olive oil for two years; then it caused a slow but marked destruction fully equal to light at the close. The tintometer for the last two years (1915–1916) showed air more destructive than light and light-moisture. Air alone was not active in producing rancidity, which was not noticeable until the sixth year. Air had no hydrolytic action but was the active factor in the decomposition of unsaturated acids and in the production of aldehyde.

Light was more active in destroying color than air or air-moisture, but according to the tintometer for the last two years it did not effect as complete destruction as air and air-moisture. Light alone was not active in producing rancidity, which was not noticeable until the sixth year, probably owing to a small amount of inclosed air. Light alone had no hydrolytic action on the glycerids or decomposing action on the unsaturated acids.

Moisture had no effect on the chromogenic bodies and did not appear a factor of any consequence in producing rancidity. Moisture caused the formation of a precipitate and a turbid oil. Moisture was the essential factor in hydrolysis and although only moderately active, gradually effected a considerable amount.

Air-light, like air-light-moisture, was the most active and effective in destroying color and in producing rancidity, which was noticeable on the second year. Air-light increased the refractive index and viscosity of the oil. Air-light had no hydrolytic action for two years; then it

gradually exceeded air-moisture and light-moisture, probably owing to the difficulty of entirely excluding moisture and at the same time permitting the entrance of air. Air-light effected more decomposition of unsaturated acids and production of aldehyde than air. Light evidently accelerated the action of air in this connection.

Air-moisture had no action on color for three years, but eventually exceeded air and equaled that of light in effectiveness. According to the tintometer, for the last two years air-moisture has exceeded light and light-moisture and has equaled that of air in destroying color. Air-moisture effected rancidity the fifth year, exceeding air and light. Air-moisture caused the formation of a slight amount of precipitate but no appreciable turbidity until the fifth year. Air-moisture effected more hydrolysis but the same amount of decomposition of unsaturated acids and formation of aldehyde as air.

Light-moisture was as effective in destroying color as light and more active than air or air-moisture. According to the tintometer, for the last two years light and light-moisture did not effect as complete destruction of color as air and air-moisture. Light-moisture caused the formation of more precipitate than moisture and a turbid oil. Light seemingly was a factor. Light-moisture effected more hydrolysis than moisture and as much as air-moisture.

Air-light-moisture, like air-light, was the most active and effective in destroying color and in producing rancidity, which was noticeable in the second year. Air-light-moisture caused the formation of apparently the most precipitate and a turbid oil. Air-light-moisture increased the refractive index and viscosity of the oil substantially the same as air-light. Air-light-moisture was the first to effect hydrolysis and exceeded all others in amount. Air-light-moisture effected the same decomposition of unsaturated acids and production of aldehyde as air-light, which greatly exceeded that of air or of air-moisture.

PRACTICAL DEDUCTIONS

From an economic standpoint air caused a slow destruction of color in olive oil, the production of rancidity, and the decomposition of unsaturated acids.

Light caused an active destruction of color and a slow production of rancidity.

Air-light caused the most active and effective destruction of color, active destruction of unsaturated acids, a rapid production of rancidity, and a slow but marked production of free fatty acids.

Moisture caused the production of a precipitate, a turbid oil, and free fatty acids.

Air-moisture practically duplicated the effect of air plus that of moisture, and light-moisture that of light plus that of moisture.

Air-light-moisture exceeded the effect of air-light plus that of moisture in the amount of free fatty acids produced; otherwise it was essentially the same.

In order to preserve olive oils in their natural state, air, light, and moisture should be excluded as completely as possible, particularly the combined action of air and light, which has proved exceedingly destructive.

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SOME BACTERIAL DISEASES OF LETTUCE

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INTRODUCTION

This paper is an attempt to classify some common bacterial softrots of lettuce (*Lactuca sativa*). The rots of lettuce, sometimes very destructive, have needed a critical study for a long time, but they have been rather neglected by plant pathologists, owing partly perhaps to their sporadic character but also partly to the fact that they are difficult to work with, as the soft, slimy character of the rotted tissues is somewhat repellent and also is prompt to invite confusing secondary invasions. Hitherto various softrots have been ascribed to bacteria, but mostly on insufficient evidence, and generally without a proper description of the supposed parasite. This paper deals with four outbreaks of lettuce rot in the United States—viz, (1) The Louisiana disease of 1915—already reported in a preliminary way by the writer (*8*)[1]; (2) the Beaufort (South Carolina) disease of 1916; (3) the Portsmouth (Virginia) disease of 1916; and (4) the Kansas disease of 1916. It also discriminates two new lettuce parasites (both Schizomycetes) and describes their morphological and physiological characters.

EARLIER LITERATURE

A short account of the literature on bacterial diseases of lettuce has been given in the paper (*8*) describing the Louisiana lettuce disease; therefore only an account of a disease of lettuce occurring in the Rio Grande Valley need be referred to.

Carpenter in 1916 gave a brief account (*9*) of an investigation of a lettuce disease occurring in the lower Rio Grande Valley. The general symptoms are those of a gradually dying plant. He describes the gross symptoms as follows: (1) A reddening of the older leaves and blanching of the younger central leaves; (2) a restricted development of newly forming leaves, accompanied by small dark-colored blister spots along the border; (3) the development of numerous lateral adventitious shoots; and (4) dry and dead small roots. Carpenter did not find any parasitic insects or fungi constantly associated with the disease. The symptoms indicated a root trouble, and he believed that the presence of alkali in the soil offered a partial explanation of the disease.

[1] Reference is made by number (italic) to "Literature Cited," p. 388.

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THE SOUTH CAROLINA LETTUCE DISEASE

The South Carolina outbreak of lettuce-rot occurred in Beaufort County, the second largest lettuce-growing district on the eastern coast of the United States, with a reputation of growing the finest quality of Big Boston head lettuce on the entire eastern coast. The South Carolina disease may be either a stem or a leaf infection (Pl. 29, A, B). In an early stage the plants are a lighter green color than the healthy ones; later the head may show rot through the center or only on the top. A general wilting of the head may occur with or without visible spots or rot. In some cases rotting is rapid; in others the heart remains sound, while the outer encircling leaves are in a bad state of decay. The diseased plants are not firm in the soil, the stem is brittle, and can be easily broken off at the surface or a little below the surface of the soil. In an early stage of disease the stem when cut across shows a blue-green color; in a later stage it is brown. If the disease attacks a young plant, no head will form. There are also cases where the stem remains sound, and only the leaves are affected, those leaves having definitely outlined spots. In others the spots have coalesced, making a darkened mass of diseased tissue. A condition of hollow stem accompanied many of the diseased plants, but there were many plants without the disease which also had the hollow stem. This hollowness of the stem at the surface of the ground or just below it may have been due to unequal growth which followed a sudden check of rapid development or of regular growth. The effect of the hollow stem was varied: Some sound heads were produced; other plants were stunted; still others formed no heads. Where there was no discoloration in these hollow stems, no bacteria were found.

The different farms showed variable amounts of the lettuce disease. On one particular farm of 9 acres there were heavy losses. A patch of 3½ acres on this farm was examined very carefully by Dr. Joseph Rosenbaum, of the Bureau of Plant Industry, and by actual count 98 per cent of the plants were diseased (Pl. 30, A). Another farm of 17 acres suffered a loss of at least 60 per cent on a conservative estimate. On other farms visited the loss was much less, varying from 1 to 15 per cent.

The direct cause of the disease was thought to be a sudden drop in temperature which occurred the middle of February, when the mercury fell to 22° F. The plants were set out in December and January from perfectly healthy seed beds.

On examining into the different cultural and soil conditions on the various Beaufort farms several facts were brought to light. The soil throughout that locality is a sandy loam; the fertilizer used was made from marsh sedge, marsh mud, and leaf mold from swamps (live-oak leaves, etc.) composted with cattle, mule, and hog manure. To be in good condition for use, this compost should be allowed to decompose for two years because of the fibrous condition of the marsh sedge and the acidity of

the leaves in the compost. Those lettuce growers who had farms entirely free from disease did not use this compost under two years' aging (Pl. 30, B). Those who had had only a small amount of the disease had used it when about 1 year old or when they found that the grass had disintegrated. These last farms were protected by windbreaks and were not so exposed to the extreme cold; consequently little damage by the disease followed. The grower who lost 98 per cent on one plot had used compost only 7 or 8 months old and not thoroughly decomposed; even at the time of the lettuce harvest in April much of the marsh grass incorporated into the compost was sticking from the soil as stubble, and the plot, which suffered severely, was unprotected by windbreaks. This piece of land had never been planted to lettuce, and the year before a crop of cowpeas (*Vigna sinensis*) had been grown on it.

Cross-sections of stems in the blue-green stage and also in the brown stage were examined microscopically and bacteria were found swarming in the tissues. No fungi were present. Both the pith and the vascular region were involved. Moderately diseased plants were darkened only in patches in the vascular region of the stem. Bacteria were found in the brown spots of the leaves on plants where the stem was not diseased. The same organism was isolated both from the stem and from the leaves, and with it the disease was reproduced repeatedly by inoculations.

Some of the soil from two different farms in Beaufort County was obtained for tests in Washington, D. C. One of these farms was the one which suffered the 98 per cent loss. Lettuce plants in seedling stage, half-grown plants, and nearly mature plants were transplanted to pots containing this supposedly diseased soil. The plants were watched carefully for nearly a month, but no trace of the disease appeared.

Samples of soils from diseased and healthy fields were examined by Dr. Oswald Schreiner, Biochemist in Charge of Soil Fertility Investigations, Bureau of Plant Industry, but he could find no significant differences between the analyses of the diseased and healthy samples. It seems reasonable to suppose that the weakened state of the plants, owing to the extreme cold, put them in a condition in which bacterial organisms could readily gain access; and the continued weakened state of the plants after the cold spell passed allowed these organisms to use the plants as a good medium for their own growth and multiplication. There must have been considerable expansion and contraction of cells during the freeze and afterwards. This was shown by the frequent occurrence of splits in the stems at the surface and just below ground. This splitting or absence of splitting might account for the presence of bacteria in some stems and not in others. And the presence of the bacterial spots on the leaves where there was no stem infection might be the result of practically the same conditions following the expansion and contraction of cells of the leaves. The lower leaves and those nearest the soil were always the most spotted.

There is one point which stands out plainly in connection with the presence in the soil of active bacteria able to produce widespread infection in a lettuce crop in from three to six weeks. The worst infected field was composed of soil heavily impregnated with a compost still in the middle stages of decomposition (Pl. 30, A), and the plants were embedded in it so loosely because of the unrotted stubble that their roots were not well protected from the cold. The field was also unprotected by windbreaks.

In Beaufort County last year (1917) a freeze in February destroyed all the lettuce plants which had been set out in the early winter; but the second crop, planted in the early spring, matured without any evidence of this bacterial disease. A lettuce crop grown last spring in the area of the 98 per cent loss was entirely free from disease. The weather conditions remained favorable for growth during the season, and the plants had no setbacks. As the soil necessary for the quick growth of lettuce must be rich in decomposed organic matter, which likewise means one rich in soil organisms, it is difficult, in a late-fall- or winter-grown crop to eliminate the chance of these organisms getting into the plants should there be temperatures low enough to weaken the plants but not to kill them. Well-decomposed organic refuse, however, presumably has fewer active organisms of parasitic types, and the chances for infection are less should unfavorable weather conditions occur.

THE VIRGINIA LETTUCE DISEASE

An outbreak of disease on lettuce grown in soil rich in decomposing organic matter occurred also in the lettuce-growing region near Portsmouth, Va., early in November, 1916, following a heavy frost. At this time the heads were of good size, well filled out, and nearly ready to harvest. The disease was indicated by a spotting mostly on the outer leaves, where the spots frequently coalesced, making dark brown, almost black, widespread areas (Pl. 31). In some cases the browning and spotting ran along the midribs, but usually the infection was worse on the blades. In other cases the tip ends of the heart leaves were stained, but there was no definite spotting as in the outer leaves. The stems and roots were not infected. Many of the heads were cut open in the field, and the hearts were found to be all right, except for an occasional stain. Cross-sections of young spots were examined under the microscope, and bacteria were found in great numbers in the tissues. It seemed evident that they had entered the plants while these were in a weakened condition, and, getting a foothold, brought on the outbreak of disease in less than three weeks after the heavy frost. The lower and outer leaves, the parts most exposed to the cold, were the ones infected.

In the Portsmouth region the writer visited four lettuce farms where the disease was present, the loss varying from 10 to 40 per cent. The growers in this section use a commercial fertilizer, but also fertilize heavily with stable manure. This year they used fresh manure, the only

kind they could obtain, and it was all bought from the same source. The grower who had the highest percentage of disease had grown sorghum and cowpeas on his land and had plowed them under two to four weeks before the lettuce was planted there. The sorghum was not decomposed in November, so that very likely it made a splendid medium for the bacteria to live and thrive in. They would then be ready to attack the lettuce when it became weakened after the frost and could no longer resist their entrance.

There was one farm, which was inaccessible at the time of inspection because of heavy rains, on which the neighboring farmers said there was no disease. The growers claimed, too, that the same cultural and soil conditions obtained on this farm as on the others.

Two different bacteria were isolated from the Virginia lettuce plants, and even from the same plant but from different spots. One organism, which formed a distinct yellow growth on potato, proved to be identical with that isolated from the South Carolina lettuce (Pl. E, fig. 3). The other organism proved to be the same as one already described as causing a serious disease of lettuce in Louisiana in 1915.(*8*) This second organism (*Bacterium viridilividum*) forms, or may form, an evanescent blue-green growth on potato (Pl. E, 1). Both organisms were inoculated into lettuce plants, and both produced disease and later were reisolated.

THE LOUISIANA LETTUCE DISEASE

The Louisiana outbreak was at Nairn, Plaquemines County, La., during the winter of 1914–1915. About 200 acres of lettuce plants were infected, and the crop was almost a total loss. The plants were nearly mature when the infection overtook them. The outer leaves of the heads were the ones most affected, being either spotted or darkened throughout. The disease did not start in the stem or roots, for the center of the heads were sound and interior parts were rotted only when the disease spread in toward the center from the outer leaves. There had been excessive rainfall in this region for three months, and the unfavorable weather was thought to be the cause of the disease. The lowland plants were affected more severely than those on the high lands. A bacterium was isolated from the spots on the leaves, and by repeated inoculations with it into healthy lettuce plants it was proved to be the organism causing the disease.

As infection started in the outer leaves, it is reasonable to suppose that pathogenic organisms were washed up from the soil on the leaves, and when the plants became weakened through unfavorable weather conditions these organisms established themselves and the plants became diseased. The name "*Bacterium viridilividum*" was given this organism, and a report made of the disease by the writer (*8*). Illustrations of the Louisiana disease are included in this paper for comparison, as none were published in the earlier paper (Pl. E, 2; Pl. 32, A, B; Pl. 35, A, B).

THE KANSAS LETTUCE DISEASE

Another lettuce disease which has proved to be of bacterial origin came to the writer's attention through Mr. L. E. Melchers, of the Kansas Experiment Station. This was a disease of greenhouse lettuce (Pl. 33, A, B), and from the material submitted a bacterium not previously reported to be infectious to lettuce was obtained. Mr. Melchers's data on varietal susceptibility, appearance of the disease, etc., which were made in the greenhouse at Manhattan, Kans., are as follows:

The day temperatures in the greenhouse where the lettuce varieties were grown ranged as closely to 70° F. as possible, while the night temperatures ranged between 50 and 56° F. The disease first appeared about December 27, 1916, when most of the varieties were about half grown. The plantings had been made from October 19 to 26. Black Seeded Simpson (leaf lettuce) was the first to show the disease and this variety became badly affected. A second planting proved just as susceptible. The leaves in rosettes that are about half grown are perhaps the most susceptible. The Improved Hansen (head lettuce) also became badly attacked; it was second in susceptibility to Black Seeded Simpson. Big Boston (head lettuce) was about as susceptible as Improved Hansen. Early Curled Simpson (leaf lettuce) was less susceptible than the three mentioned varieties. Vaughan's All Season (head lettuce) only showed slight infection. Grand Rapids (leaf lettuce) seemed immune to attack, the disease did not appear on this variety.

The symptoms of this disease are quite striking. At first a slight marginal wilting takes place in more or less localized areas on leaves about the same age in the same whorl. The areas attacked in the leaf margins may vary from mere specks to areas two or three centimeters long and by coalescing, areas extending seven centimeters have been observed. The diseased areas scarcely ever extend more than three centimeters down the leaf, generally less than this. On the older leaves the most common sign is the wilting of the tips. The areas affected lop over and gradually become dry. The vascular tissues at this stage frequently show a distinct browning. In a few days the affected areas turn brown, tan, reddish, and sometimes black; the tissues become papery and dry in texture. This disease does not progress down the entire leaf, but ceases development after it extends a short way. It does not cause a rot or soft decay of lettuce but mars its appearance, so that it is not salable. Frequently the wilting symptoms do not appear until after a discoloration of the vascular system is noticed. Often brownish, water-soaked areas are seen and these tissues are turgid at the time. A speckled appearance is sometimes observed below the margins. This is caused by a slight discoloration of the vascular system in localized regions.

In a letter Mr. Melchers stated that he felt satisfied the infection comes from the soil and is carried to the plants by watering and by currents of air, that he suspected the disease was of bacterial origin, and that the organism gets its start by entering the younger leaves at the tips, where moisture is likely to remain for a longer time.

Spraying inoculations made with the organism isolated by the writer from the Manhattan plants proved that the organism enters the young leaves at the tips if they are kept moist. No wounding of the plants is necessary; poor ventilation is the only requisite after the plants are sprayed with water suspensions of young agar cultures.

Specimens of a lettuce disease which occurred in Hutchinson, Kans., were also sent to the writer by Mr. Melchers. This disease affected the Grand Rapids (loose-leaf) lettuce, the only variety at Manhattan not affected with the marginal disease. These plants had tiny irregular spots all over the blade and in the midrib; they were yellowish red in color, almost like rust spots (Pl. 34). In some places the spots coalesced. As in the Manhattan disease, bacteria were found swarming in the diseased places when cross-sections of those areas were examined microscopically. An organism was isolated from the Hutchinson plants which proved to be identical with the Manhattan organism. Inoculations proved this organism to be infectious. Besides the yellowish-red speckling, marginal infection also occurred. Successful inoculations were made into the Boston Head and Golden Queen varieties.

No further spread of the disease in this Hutchinson greenhouse was reported. The infection in all probability arose through an accident to the subirrigation system, for it was learned that in watering the plants through one of the tiles, the hose in some way worked out and threw the water for about half an hour over that part of the greenhouse where the disease occurred later. Because the disease did not occur on other, more susceptible varieties, and because the water from the disrupted irrigation system did not deluge those varieties, the accident is quite significant

It is the writer's opinion that the organisms dried up on the leaves of the Grand Rapids lettuce at Manhattan before they had a chance to enter them; consequently that variety did not become infected at the same time as the others in the same house. The very nature of loose-leaf varieties is such that there is better ventilation between the leaves; and if the young rosette at the center dries quickly after watering, there is little chance for the bacteria to get inside the leaf, since laboratory tests of this organism have shown that it is killed very readily by drying. In the case at Hutchinson where the disease occurred on the Grand Rapids variety, there was little chance for the drying out of any part of a leaf outside or toward the center of the head while the irrigation system was out of order. It is likely the bacteria were washed from the soil into the breathing pores of the leaves, for their presence later in irregular spots all through the blades shows that they took advantage of this condition, which was not confined to the margins of the moist center leaves.

ISOLATIONS AND INOCULATIONS WITH ORGANISM FROM THE SOUTH CAROLINA LETTUCE

The organism was isolated from the interior of the stem of the diseased South Carolina plants which showed the brown discoloration and also from the young spots on the leaves. The leaf portions were sterilized for one minute and the stems for two minutes in mercuric chlorid (1 : 1,000), washed in sterile water, mashed up in bouillon, and agar

plates were poured. The surface colonies appeared in from two to four days. They are at first a light-cream color, round, wet-shining, with fine surface markings. The margin is entire, with light and dark areas in an hourglass arrangement when viewed by transmitted light. When older, the colonies are yellow, without surface markings (Pl. 35, C).

Inoculations were made by spraying mature lettuce plants with pure cultures of the bacterium suspended in water (24- to 48-hour agar slants washed off in sterile water) and by pricking some of the leaves with a sterile needle. Those leaves of the older plants which were punctured became infected readily. Inoculations were made also by smearing the bacterial slime on the leaves and stem, and then puncturing the smeared places with a fine sterile needle (Pl. 36, A, B). Plants beginning to head or already headed became diseased readily, and those about to send up a seed stalk always showed the worst infection (Pl. 36, D, E). Young plants were only slightly affected, and usually recovered. Plants sprayed but not punctured rarely became infected. Repeatedly inoculations were made successfully; then the organism was reisolated; the reisolation colonies proved likewise to be infectious. The original colony kept growing on artificial media was still infectious a year after isolation.

The organism was inoculated into cabbage (*Brassica oleracea capitata*) in order to compare it with inoculations with *Bacterium campestre*. No infection followed with the lettuce organism, but *Bact. campestre* infected the cabbage readily. It was thought, too, that this lettuce organism might prove infectious to the heart of celery; therefore inoculations were made twice into young plants by spraying and by punctures, but with negative results. Nearly mature celery plants were treated in the same way with the same results.

DESCRIPTION OF THE SOUTH CAROLINA ORGANISM

The organism is a bacterium, a short rod with rounded ends, occurring singly or in pairs, occasionally in short chains. In stained host tissue the measurements of single rods vary from 0.62 to 1.04 μ long, and 0.42 to 0.83 μ wide (Pl. 41, A). Grown for one day on beef agar and stained with Loeffler's flagella stain, they vary from 0.62 to 1.24 μ long and 0.42 to 0.83 μ wide.

The organism is not actively motile; often in the sections of fresh tissue in which the bacteria occurred in numbers very little or no motion could be detected on microscopical examination. The motility was demonstrated better in young agar cultures. The flagella are polar, varying from one to several at each pole, but most commonly one at one pole. They were stained by Casares-Gil's flagella stain (Pl. 41, B).

Capsules were stained by Van Ermengem's flagella stain. The absence of spores was tested by staining and also by heating old live bouillon cultures. The tests were negative.

Pseudozoogloeæ occur and are composed of masses of short and long chains hanging together by a network of gelatinous threads. No long filaments or queer-shaped cells were noted. Swollen cells and others much reduced in size were noted in old cultures grown under low-temperature and high-temperature conditions, acid-media cultures, and cultures with sodium chlorid.

BEHAVIOR TOWARD STAINS

The organism stains readily and uniformly in the common anilin stains, such as gentian violet, methyl violet, dahlia, and carbol fuchsin. It is Gram-negative, and is not acid-fast.

CULTURAL CHARACTERS

Sterile potato cylinders proved to be a very good medium for this organism, the color of the bacterial slime being a bright yellow. Beef bouillon and litmus milk were favorable media for prolonged growth.

BEEF-AGAR PLATES.—The colonies on peptonized beef-agar plates (+15 Fuller's scale) are visible in 24 to 48 hours, room temperature 20° to 25° C., when poured from a young bouillon culture. They are at first a light-cream color, smooth, thin, round, edge entire with light and dark areas in a sort of hourglass arrangement. These areas disappear when colonies are 2 to 3 days old. Most of the colonies are cream color[1] throughout; some have blue areas or a ring of blue color with a cream center when viewed in transmitted light. When they are 4 to 5 days old, they are all a deep cream-yellow color, and from 3 to 6 mm. in diameter. Buried colonies are round or elliptical (Pl. 35, C).

AGAR STROKE.—In two days at 25° to 28° C. there is a moderate, cream-yellow growth, thin, flat, spreading, opaque, smooth, entire margin, viscid, yellowish in condensation water. Crystals abundant in three days. Growth remains moderate. Agar does not change color.

AGAR STAB.—There is very little growth in two days; in three days a fair amount of surface growth, faint filiform growth along line of puncture. Color of growth, honey-yellow. Crystals occur in agar just below the surface. In 14 days the color of the growth is old gold, and the crystals extend down into the agar near the puncture. In 30 days the growth is mustard color, and prismatic crystals occur throughout the agar.

BEEF BOUILLON.—Peptonized +15 beef bouillon is clouded faintly in 2 days at room temperature (25° to 28° C.). At 3 days most of the growth is at the surface until tube is agitated, and then the growth falls in tiny filmy flakes. No color change. In 8 to 10 days there is an interrupted pellicle, and the bouillon is yellowish, with a viscid sediment. In 41 days the pellicle is still incomplete, with long filaments hanging down in the medium. There is usually a yellow rim and a heavy viscid sediment in the bottom; the rest of the culture is usually clear and in color is old gold.

NEUTRAL BEEF BOUILLON.—In 3 days there is a faint growth at a temperature of 22° to 26° C., in 5 days a fair growth, and at 10 days a pellicle which sinks in long strands on handling the tube.

BOUILLON CONTAINING SODIUM CHLORID.—In 4 days there is slight growth in neutral beef bouillon containing 3 per cent of sodium chlorid. In 7 days there is a good growth. No growth occurs in the bouillon to which 4 per cent of sodium chlorid has been added.

[1] The colors mentioned in this paper are given according to Ridgway (RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., col. pl. Washington, D. C., 1912).

BOUILLON OVER CHLOROFORM.—Growth occurs, but is retarded. Only a slight clouding occurs in 7 to 8 days, and in 30 days the growth is still slight.

USCHINSKY'S SOLUTION.—The organism does not grow readily in this medium. There is a slight growth in 4 days. In 30 days it is still slight, with only a faint white clouding. This was repeated seven times. No growth occurred in three of the tests.

COHN'S SOLUTION.—The growth is very faint, and often does not occur. Out of eight tests definite growth occurred three times, faint growth twice, questionable twice, and once not at all. One infectious colony of the two used throughout this work might grow in one lot of media, while the other might not.

FERMI'S SOLUTION.—No growth occurred.

STERILE MILK.—In 5 days the medium is clear to a depth of only 3 mm. below the surface. In 7 to 9 days it is about half clear, with no acid coagulation, but with a heavy curdlike precipitate. There is a slow separation of curd and whey by 11 days. At 16 days there is no yellow color in the whey or the precipitate, but the bacterial growth at the surface is yellow. In 49 days very little curd is left, and this is present in little balls. The color of the whey has changed to yellow again, a light-orange yellow.

LITMUS MILK.—There is a slow reduction. The color changes in rings, a deep blue at the top, shading down to lilac litmus color at the bottom. In 5 days one-third of the medium from the top down is clear, and is darker blue, with a yellow bacterial precipitate. In 7 days a curd has formed; there are still three shades in the medium, anthracene-purple at the top, shading to a brownish at the bottom. At 18 days the purple color has disappeared, and the entire medium is a light brown; the curd is in suspension. After a month the medium is light brown throughout, except at the very surface, where it is purple. There is a viscid mixture of curd and bacteria through half the medium, numerous balls of white curd floating in this viscid mixture.

NUTRIENT GELATIN.—The colonies are slow in appearing on peptone gelatin (+10) plates at 12° to 15° C. In two tests made the colonies did not appear before 7 to 9 days. Even when the plates are not thickly sown, the colonies do not develop a diameter larger than 4 mm. They are yellow, round, shining, and thicker than beef-agar colonies. Buried colonies are both round and oval. Liquefaction begins when the colonies are 3 days old, in little cups around them, continuing slowly. When they are 16 days old, the gelatin of the thinly sown plates has not entirely liquefied.

The stab cultures liquefy slowly also at a temperature of 12° to 15° C. In 2 days there is slight growth on the surface and along the line of puncture, but no liquefaction. In 9 days there is a slight crateriform liquefaction, and in 12 days the liquefaction has reached almost across the surface of the gelatin. In 30 days 1.5 cm. of the medium are liquefied, in 40 days one-half, and in 57 days all except one-sixth at the bottom of the tube.

STEAMED POTATO CYLINDERS.—There is abundant growth in 2 days at a temperature of 25° C. The growth is smooth, thick, viscid, shining; the color is empire-yellow (Pl. E). In 14 days the growth is a dark olive-buff, and the medium has changed to a grayish brown.

There is a feeble diastasic action on the starch.

OTHER CULTURAL FEATURES OF THE ORGANISM

INDOL.—There is slight production of indol in 1 per cent peptone-water cultures 10 days old. It is still slight when the cultures are 16 to 20 days old.

NITRATES.—Nitrates are not reduced. Tests were made when nitrate bouillon cultures were 7 and 17 days old.

AMMONIA PRODUCTION.—Moderate.

HYDROGEN SULPHID.—Hydrogen sulphid is produced. Cultures of beef agar, beef bouillon, milk, and potato cylinders were tested by hanging lead-acetate paper in

the tubes where the transfers were made. The paper became well blackened in every case.

TOLERATION OF ACIDS

Tests were made with tartaric, malic, and citric acids, and it was found that the organism is most sensitive to the presence of small quantities of citric acid. There was a good growth in neutral beef bouillon with 0.1 per cent of tartaric acid, which titrated +23 on Fuller's scale, but no growth at all where 0.2 per cent of tartaric acid was added. There was good growth also in neutral beef bouillon to which 0.1 per cent of malic acid was added, which titrated +25 on Fuller's scale. There was no growth in the bouillon to which 0.2 per cent of malic acid was added. Three tests were made with neutral beef bouillon to which 0.1 per cent of citric acid was added, but growth occurred only once, in which the medium titrated +17. The negative tests titrated a little higher.

TOLERATION OF SODIUM HYDROXID

The organism tolerates sodium hydroxid to −25 on Fuller's scale. Tests were made in beef bouillon containing sodium hydroxid titrating −20, −25, −30, −35, and −40. In 2 days there was a slight clouding in −20 and in −25, but none in −30, −35, or −40.

TEMPERATURE RELATIONS

THERMAL DEATH POINT.—When transfers are made from a well-clouded bouillon culture of 24 hours and kept at 52° C. in a water bath for 10 minutes, no growth occurs. This test was repeated many times. Sometimes growth occurred at 51°. The thermal death point lies, therefore, between 51° and 52°.

MAXIMUM TEMPERATURE.—The maximum temperature for growth is 35° C.

MINIMUM TEMPERATURE.—The minimum temperature for growth is below 0° C.

OPTIMUM TEMPERATURE.—The optimum temperature is 26° to 28° C.

Beef agar and bouillon cultures were used for the three preceding temperature tests.

GAS FORMATION.—The organism is aerobic and does not form gas. It was tested in fermentation tubes in the presence of each of the following carbon compounds: Glycerin, dextrose, lactose, saccharose, maltose, and mannit, 1 per cent of these being added to a 1 per cent water solution of Witte's peptone. No gas formed in any of the tubes, and no growth took place in the closed arm of the tubes. However, growth occurred in the open end of each tube. In the test for acid and alkaline reactions with neutral litmus paper all showed alkaline reactions.

FURTHER TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. Tests were made by placing agar and bouillon transfers in a specially devised jar from which the oxygen was removed in the following way: 40 gm. of pyrogallic acid were dissolved in potassium hydroxid (35 gm. to 350 c. c. of water), and this mixture was placed uncovered in a bottle in the jar with the cultures. The top of the jar was covered; then another cover inserted in a bed of mercury was placed over the whole. The experiment was watched carefully, yet no growth could be detected until the cultures were removed at the end of two weeks, when one of the bouillon cultures was found to have developed a few threads of filamentous growth extending from the surface into the medium, but no clouding occurred. No growth occurred in the stab cultures, a trace of growth in one tube not being considered significant. The control cultures showed good growth in one day. After removal from the jar growth took place in the bouillon cultures, but there was none in the agar.

RELATION TO LIGHT

The organism is not very sensitive to sunlight. Thinly sown agar plates were exposed bottom side up on cracked ice, one side of the plate being covered with black paper. Midday on bright sunny days in early winter was taken for the test. The temperature of the ice bag was 8° to 10° C. A 40-minute exposure did not kill the organism, and in some tests even a few colonies appeared after 50 minutes' exposure; but no colonies appeared on those plates exposed for 60 minutes.

RELATION TO MOISTURE

The organism is not killed very readily by drying. Drops of a 1-day-old bouillon culture were transferred to sterile cover glasses in a petri dish, and the dish was placed in the dark. The temperature of the room during the days of this test was 25° to 30° C. When kept for two days, and dropped in tubes of bouillon, growth occurred; but no growth occurred in those tubes which received covers on which the organism had been drying for three days.

VITALITY IN CULTURE MEDIA

This bacterium lives for more than a year in liquid culture media when cultures are kept in the refrigerator at temperatures of 12° to 15° C., and do not evaporate readily. At room temperatures (20° to 25°) it lives from two to three months. Milk and litmus milk are the most favorable media for continued growth at these temperatures. In two months the organism is dead in bouillon and on potato cylinders.

LOSS OF VIRULENCE

No loss of virulence was noticed when inoculations were made within eight months to a year after isolation.

GROUP NUMBER

According to the descriptive chart of the Society of American Bacteriologists, the group number is 211.3332523.

The name "*Bacterium vitians*, n. sp.," is suggested for this organism.

BRIEF TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium vitians, n. sp.

A short motile rod with rounded ends, flagella bipolar, but usually one at one pole; capsules, pseudozoogloeæ, no spores, involution forms rare and few types, aerobic; agar colonies, light-cream color, smooth, thin, round, light and dark areas in an hourglass arrangement when young; when older, shading disappears, and all are cream-yellow. Growth on potato cylinders is abundant, bright yellow; produces alkaline reaction in litmus milk, with a gradual separation of the whey from the curd, curd partly digested; liquefies gelatin slowly; produces ammonia, hydrogen sulphid, indol (slight); does not reduce nitrates; feeble diastasic action on potato starch; grows in Uschinsky's solution; grows feebly or not at all in Cohn's solution; thermal death point 51° to 52° C. Maximum temperature for growth 35° C., minimum below 0° C., optimum 26° to 28° C. Vitality two months to over a year in liquid media, depending on temperature and evaporation. Is Gram negative, and is not acid-fast; stains readily with basic anilin dyes. Not killed very readily by drying, not very sensitive to sunlight; slight toleration of acids and alkalies (tolerates tartaric in neutral beef bouillon to +23 Fuller's scale, malic +25 Fuller's scale, citric +17; tolerates sodium hydroxid in beef bouillon to −25); retains its virulence over one year.

ISOLATIONS AND INOCULATIONS WITH ORGANISMS FROM VIRGINIA LETTUCE

Two organisms were isolated from the spots in the diseased plants from the lettuce-growing sections along Hampton Roads, Virginia: one (*8*) the Louisiana organism, *Bacterium viridilividum* (Pl. 35, D), and the other the South Carolina organism, *Bacterium vitians*. Isolations were made from plants from three different farms, and whatever skepticism there might have been at first because of the presence of two distinct pathogenic organisms was dispelled when the two familiar colonies persisted in appearing on the plates.

The isolations of *Bact. viridilividum* produced spotting of the leaves when inoculated into greenhouse plants (Pl. 37, A). The isolation of *Bact. vitians* also produced spotting and rotting of leaves when inoculated into greenhouse plants (Pl. 38), and likewise the typical stem disease when inoculated into the stems (Pl. 37, B). There was no natural infection of the stems in the diseased lettuce from the Virginia fields, but the inoculations in the stem were made to prove the full pathogenicity of the organism as compared with that isolated from the

South Carolina plants. In South Carolina the stem infection was more prevalent by far than the leaf spotting alone. *Bact. viridilividum* from the Virginia plants became blue-green on sterile potato cylinders (Pl. E, 1) the same as *Bact. viridilividum* from Louisiana (Pl. E, 2), but like the Louisiana isolation, the color is fleeting, and frequently there are infectious colonies which will not produce the blue-green color on potato, but which agree in other cultural features.

Morphological and cultural tests were made with *Bact. vitians* from the two sources, South Carolina and Virginia, and no doubt remains as to their identity.

ISOLATIONS AND INOCULATIONS WITH ORGANISM FROM THE KANSAS LETTUCE

ISOLATION OF THE ORGANISM

Pieces of the browned marginal areas from the diseased material from Manhattan, Kans., and of the small irregular reddish spots from the material from Hutchinson were used for isolating. The pieces were immersed in mercuric chlorid (1:1,000), one test for 2 minutes and another for 3 minutes, washed in sterile water, and mashed up in bouillon. Surface colonies appeared in two days, thin, bluish white, round, shining, some slightly convoluted, most with a smooth surface. The color changes to cream, then yellowish, and the agar becomes a brilliant green. The colonies range from 2 to 7 mm. in diameter when several days old.

INOCULATIONS

Inoculations with the organism isolated from the Kansas lettuce were made by spraying water suspensions of young agar cultures on young and half-grown lettuce plants. No wounding was necessary to produce infection. The margins of the inner whorl of leaves became dark brown, almost black, in 24 to 48 hours; the outer leaves were not infected. At first these brown margins were soft, but in a few days they became dry and papery, with a brown discoloration extending farther in the veins and veinlets. This condition had been noted on the infected leaves received from Kansas (Pl. 39, A, B). The infected margins were from 0.5 to 1.5 cm. in width, rarely wider. A very tiny curled-up leaf might be entirely browned. Some of the infected places, but not all, first showed as little reddish and brownish spots, and the veins showed darkening before the parenchyma.

Inoculations were made on plants growing in the open bed and also in pots, which were placed in infection cages where there was plenty of moisture (Pl. 40, A, B). Occasionally there would be a plant which resisted infection in the open bed, but scarcely ever one in the infection cage. The temperature of the greenhouse did not seem to have so

much effect on the results of the inoculation experiments as the ventilation and moisture.

The inner whorl of leaves was the part of the plant usually infected. No mature closed heads were inoculated, but occasionally in infection-cage experiments some of the older leaves had numerous red speckled areas, which, on examination, proved to be filled with bacteria.

One of the inoculation tests was made in two greenhouses during the winter when the temperature of one was 8 to 10 degrees lower than that of the other. The plants were placed in infection cages, where there would be little ventilation and high humidity. The disease took readily in both houses. This test was followed up in the summer, when both houses were practically of the same temperature. The plants were grown in open beds, those in one house being set farther apart and kept better ventilated than the other one. The plants of both houses were inoculated by spraying them with the same cultures. The infection produced in the well-ventilated house was almost negligible, while the usual blackened margins of the inner rosette of leaves occurred on the close-set plants in the poorly ventilated greenhouse. The organism was reisolated from the diseased margins, and on inoculating with the colonies so obtained, the disease was again produced.

This disease of lettuce need not be confused with the browning of margins of lettuce leaves due to tipburn, or sunscald, for the brown of tipburn is a much lighter color.

The hearts of young and old celery plants were inoculated with the Kansas organism by spraying, also by smearing the bacterial slime on the leaves and then puncturing them. No infection followed on either young or old plants.

DESCRIPTION OF THE KANSAS ORGANISM

The organism is a bacterium motile by means of polar flagella, one or two at each pole, a few noted with three at a pole Casares-Gil's flagella stain, (Pl. 41, E). It is a short rod rounded at the ends, occurring in short chains or singly. Stained with carbol fuchsin in the leaf it is 0.83 to 1.66 μ long and 0.83 to 1.25 μ wide, the majority being 1.45 μ long and 0.83 μ wide. Grown on beef agar for 24 hours and stained with gentian-violet, it is 0.83 to 1.87 μ long and 0.42 to 0.83 μ wide. Stained with carbol fuchsin, same age, it is 1.25 to 2.08 μ long and 0.42 to 0.83 μ wide.

Capsules were stained by Ribbert's capsule stain (Pl. 41, D).

Endospores are not produced. Tests were made by boiling several liquid cultures of different ages for 3 minutes; also heating others to 80° C. for 20 minutes. Transfers were made in each case before and after boiling. Before heating and boiling all cultures were alive, as growth took place in the transfers, but none took place in transfers made from cultures after they had been boiled or heated. The organism forms

pseudozoogloœæ, grows in clumps very quickly in some of the liquid media—for example, in beef bouillon—and when first isolated it produces a very offensive odor. About 4 or 5 months after isolation, the odor was considerably less, and in 8 months none of it could be detected.

BEHAVIOR TOWARD STAINS

The organism stains very readily in methyl violet, carbol fuchsin, safranin, and dahlia. It is Gram-negative, and is not acid-fast.

CULTURAL CHARACTERS

This organism grows readily in most of the media, and is a much more rapid grower than either *Bact. vitians* or *Bact. viridilividum*. It belongs to the green fluorescent group. Beef bouillon (+15) is a very successful medium. In fact, growth took place too rapidly in this medium for many tests where a thinly clouded culture was needed, and low temperatures had to be used as soon as the transfer was made, or an acid bouillon or a 5 per cent sodium-chlorid bouillon used to delay the too rapid growth.

BEEF-AGAR PLATES.—Colonies appear in from 24 to 48 hours when poured from a young bouillon culture. The temperature of the room may vary from 22° to 30° C. At first the surface colonies are a faint bluish white, then cream color, and later a yellowish color; they vary in size from 3 to 7 mm. in diameter, are round, smooth, occasionally convoluted, thin; at first there are fine surface markings which disappear as the colonies get older (Pl. 35, E). The agar becomes yellow-green.

AGAR STROKE.—The growth in 2 days is thin, spreading, yellow. The agar just below the surface is a viridine green. The surface of growth is rather finely papillate than smooth. In 10 days all the agar is colored Javel green; the growth is abundant, glistening, viscid.

AGAR STAB.—The surface growth is rapid, but growth is feeble along the stab. At 2 days it is cream-colored with no discoloration of agar. In 7 days growth is yellow and nearly covers the surface; the agar is viridine green just below the growth at the surface and along the stab. Crystals appear below the surface.

BEEF BOUILLON.—Peptonized +15 beef bouillon is clouded very readily in 18 to 24 hours at temperatures of 20° to 30° C. At temperatures of 5° to 11° it is thinly clouded in 24 hours. Usually a pellicle which breaks up easily has developed in 3 days; the bouillon is viridine green at the surface and for about 2 cm. down. In 6 days the upper part of the medium is apple-green, and besides the pellicle there is a white precipitate. In 30 days there is a heavy viscid growth at the bottom of the tube; the bouillon is clear; and the color is olive-ocher.

BOUILLON OVER CHLOROFORM.—Chloroform does not retard the growth. Clouding takes place in 24 hours, and a heavy growth follows.

BOUILLON CONTAINING SODIUM CHLORID.—There is good growth at once in neutral beef bouillon containing 3 per cent of sodium chlorid, a slight retardation but heavy growth in that containing 5 per cent, only a fair amount of growth in the bouillon containing 6 per cent, and none at all in that containing 8 per cent.

GELATIN PLATES.—Colonies are up in 4 days on +10 beef-peptone gelatin plates at 11° to 15° C.; are 1 mm. in diameter, a deep-cream color, bluish in transmitted light, with margins slightly indented. Liquefaction (cup-shaped) begins when colonies are 2 days old. No color change occurs at this age. When colonies are 5 days old, they are 2 to 4 mm. in diameter and the gelatin is greened around them for some distance. This color is mineral-green. At 15 days the liquefaction is still cup-shaped around colonies, but the green color has spread through the entire gelatin. At 25 days the gelatin is not all liquefied.

GELATIN STAB.—In beef-peptone gelatin +10 stabs liquefaction begins in from 2 to 4 days in a crateriform way at a temperature of 11° to 15° C. Growth is good at the surface, feeble along the stab; there is a faint green color at the surface. In 6 days gelatin is liquefied three-fourths across the surface of the stab, the color just below being viridine green. In 15 days the surface of the gelatin is liquefied straight across for a depth of 1 cm. and the color, which extends halfway down the tube, is yellow-green, a brighter green than that of the plates.

USCHINSKY'S SOLUTION.—There is heavy clouding in 24 hours. In 3 days the upper half of the medium is greened. In 5 days there is a heavy pellicle, and the medium throughout has become Veronese-green.

COHN'S SOLUTION.—The organism does not grow in Cohn's solution.

FERMI'S SOLUTION.—No growth.

STERILE MILK.—In 3 days about one-third of the medium is cleared and is a sea-foam-green color; the rest is a soft curd. In 20 days the milk is all cleared with curd in suspension. The color is citron-green. In 47 days the milk is a darker color, lime-green. The curd is in suspension in bottom of tube; the rest of the liquid is clear.

LITMUS MILK.—In 2 days the color of the medium has begun to change in rings, a turbid reddish color at the top for 4 mm., then 2 cm. of a lighter shade below, and next the ring of lilac-litmus color. A pellicle is on the surface; there is no coagulation. In 4 days none of the original lilac-litmus color is left; the medium is clear and half of it is reddish brown (dark vinaceous drab); the rest is a lighter color. At 7 days the upper half of the medium has more red color in it—is dark mineral-red. The rest of the milk is a reddish-tan color. There is a heavy pellicle and a soft curd. In 25 days the medium has changed to a dark-blue shade, is blue-violet-black.

STEAMED POTATO CYLINDERS.—There is a thin watery growth covering the surface of the cylinder in one day. Plate E, 5, gives the appearance at the end of the second day. In 4 days this growth is a pinkish-

tan color, but is still thin, and the medium is unchanged. In 13 to 16 days (Pl. E, 6) the color of the growth is warm-buff and the potato has darkened slightly. In 30 days there is no further change. There is feeble diastasic action on potato starch.

OTHER CULTURAL FEATURES OF THE ORGANISM

INDOL.—No indol is produced.

NITRATES.—There is a good reduction of nitrates. Tests were made with nitrate bouillon cultures in which the organism grew very well. One c. c. potato-starch solution was added to each culture; then one c. c. of a fresh potassium-iodid solution (1:250), after which five drops of dilute sulphuric acid (2:1) were added. A dark-blue color, indicating reduction, followed immediately.

HYDROGEN SULPHID.—No hydrogen sulphid was detected.

AMMONIA.—The organism produces ammonia. Cultures of bouillon agar, and Uschinsky's solution (2 to 6 weeks old) were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. A brownish-red color appeared on the filter paper immediately, indicating the presence of ammonia.

TOLERATION OF ACIDS.—There is a moderate toleration of citric, malic, and oxalic acids. In the tests these acids were added to neutral beef bouillon.

A good growth occurred in two days in the bouillon containing citric acid titrating +37 on Fuller's scale, but no growth occurred in that titrating +39.

With malic acid there was a good growth in four days in the solution titrating +38, but none occurred in that titrating +40.

With oxalic acid there was a good clouding in +37 in six days, but no growth in +40.

TOLERATION OF SODIUM HYDROXID.—The toleration of sodium hydroxid by this bacterium is moderate. There is heavy clouding and pellicle in −20 beef bouillon, fair clouding in −25, and faint clouding in −30 in two days. No growth occurs in −40.

GAS FORMATION.—The organism is aerobic, and does not form gas. Tests were made in fermentation tubes with water containing 1 per cent of Witte's peptone to which was added 1 per cent of each of the following carbon compounds: Dextrose, lactose, saccharose, maltose, glycerin, and mannit. Growth occurred in the open end of the tubes, but none took place in the closed end and no gas was produced. Dextrose and saccharose gave an acid test with litmus after the organism had been growing in the tubes for six weeks. Glycerin, maltose, mannit, and lactose gave an alkaline test.

FURTHER TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. A special flask from which the oxygen had been absorbed by a mixture of pyrogallic acid and potassium hydroxid was used for the test (described on p. 374). Transfers of the organism were made to +15 bouillon and beef-agar stabs and placed in the jar. At the end of two weeks, when they were removed, there was a mere trace of growth in the stabs, and there was a thin pellicle on the top of one of the bouillon cultures. But there was no clouding and no green color so characteristic of this organism. The controls showed good growth in one day. The organism is a rapid grower, and it is likely that the oxygen in the culture tubes was not absorbed promptly enough to exclude all growth. Seven days after removal from the jar the bouillon cultures were clouded, but no growth had taken place in the agar.

TEMPERATURE RELATIONS

THERMAL DEATH POINT.—The thermal death point lies between 52° and 53° C. when transfers are made from a thinly clouded culture which does not contain clumps of bacteria and they are kept in the water bath for 10 minutes. If an 18 to 24 hour old +15 bouillon culture which is densely clouded is used there will be growth when the transfers are subjected to 55° and 56° C. for 10 minutes. This is because of the tiny masses of bacteria which hold together in clumps, the inner ones of which are somewhat protected.

MAXIMUM TEMPERATURE.—The maximum temperature for growth is 38° C.

MINIMUM TEMPERATURE.—The minimum temperature for growth is below 0° C.

OPTIMUM TEMPERATURE.—The optimum temperature for growth is 25° to 26° C.

The medium used for these three preceding temperature tests was +15 peptone-beef bouillon.

RELATION TO LIGHT

The organism is not particularly sensitive to sunlight. The different sets of plates for this test were poured from bouillon cultures that were not heavily clouded. The plates were exposed to bright sunlight at noonday in June and July, one-half of each plate being covered with carbon paper and placed, bottom side up, on sacks of cracked ice, the temperature of the bag being 8° to 14° C. No colonies appeared on the uncovered side of the plates exposed for 40 minutes while from 50 to 70 colonies appeared on the covered sides. In four separate tests colonies appeared twice on the exposed side of 35-minute plates, and twice none appeared. On 30-minute plates 1 to 10 colonies appeared on the exposed sides

to over 50 on the covered sides. On 25-minute plates from 3 to 5 colonies appeared on the exposed sides, while more than 50 appeared on the covered sides. Many colonies appeared on the exposed sides of 15 and 20 minute plates.

RELATION TO MOISTURE

The organism is killed readily by drying. When a +15 bouillon transfer is kept in the refrigerator for one day at 11° to 12° C., there is clouding but no heavy growth. If transfers of drops are made to sterile cover glasses from such a culture and the cover glasses kept in the dark at 25° to 27°, the bacteria die in five hours but are still alive at three hours. The test was made by dropping them in tubes of beef bouillon after those intervals had elapsed.

If a 1-day-old heavily clouded +15 bouillon culture, which contains the clumps of bacteria is used, drying does not take place so readily, and cover glasses in the dark at 24° will still have live bacteria on them after drying for six days.

VITALITY IN CULTURE MEDIA

The organism lives for 5 months in beef-agar stabs and more than six months in beef bouillon and sterile milk when kept at room temperatures varying from 24° to 30° C. If evaporation is such that the cultures dry down, they will die before this time has elapsed Cultures kept in the refrigerator will live from 9 to 10 months.

LOSS OF VIRULENCE

The organism is still virulent at the time of writing, more than a year after isolation.

GROUP NUMBER

According to the descriptive chart of the Society of American Bacteriologists, the group number is 211.2323123.

The name "*Bacterium marginale*, n. sp." is suggested.

BRIEF TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium marginale, n. sp.

It is a short rod with rounded ends; flagella 1 to 3 bipolar, capsules; pseudozoogloeæ; no spores; few involution forms noted; aerobic; agar colonies cream-colored when young, yellow when mature and the agar a brilliant green; clouds bouillon very heavily in 24 hours at 20° to 30° C., and in six days the medium is apple-green; growth on potato cylinders is scanty and dirty cream-colored (Pl. E, 5, 6); later it is a warm-buff. The potato darkens slowly; the diastasic action is feeble; liquefies gelatin slowly; produces ammonia; fluorescence green; reduces nitrates; does not produce indol nor hydrogen sulphid; grows in Uschinsky's but not in Cohn's or Fermi's solution; optimum temperature 25° to 26°; maximum 38°; minimum below 0°. Thermal death point 52° to 53° (under conditions stated); vitality at room

temperature six months in liquid media; stains readily with basic anilin dyes; is Gram-negative; not acid-fast; not very sensitive to sodium chlorid (tolerates 6 + per cent); moderate toleration of acids and alkalies (tolerates oxalic to + 37 on Fuller's scale; malic + 38; citric + 37; tolerates sodium hydroxid in beef bouillon to −30 Fuller's scale); is killed readily by drying; not very sensitive to sunlight; retains its virulence for more than one year.

CONTROL OF LETTUCE DISEASES

THE SOUTH CAROLINA AND VIRGINIA DISEASE

So far as known, the *Bacterium vitians* gets into the field lettuce only when it is in a weakened state owing to sudden cold weather which is not cold enough to kill the plants. The treatment recommended is the use of thoroughly decomposed green manure and well-seasoned stable manure in which tissue-disintegrating bacteria have practically finished their work. The bacteria then present in the soil are not active and the plant, though weakened by sudden severe cold, may regain its stability and be able to resist their entrance.

The use of satisfactory windbreaks is obvious.

THE KANSAS DISEASE

As *Bacterium marginale* is a soil organism also, care should be taken in watering the plants in the greenhouses that the roots only of lettuce are watered. Soil should not be washed up nor spattered on the leaves. Subirrigation is a safeguard.

Good ventilation will almost, if not entirely, prevent the disease.

SUMMARY

Two new bacterial diseases of lettuce are described in this paper. One occurred in South Carolina and in Virginia the same year both on winter and late fall crops grown out of doors. The other is a disease of greenhouse-grown plants in Kansas.

The South Carolina disease occurred in the stems and roots and less frequently on the leaves, following a sudden drop in temperature in February. The Virginia disease occurred on the leaves only and followed a heavy frost in October. An infectious organism identical with the South Carolina bacterium was isolated.

Inoculations were made with the bacterium isolated from the South Carolina and Virginia lettuce, and this organism from both sources proved to be infectious to both stem and leaves of lettuce. The name "*Bacterium vitians*" is suggested for this organism.

Besides this organism another was present in the Virginia lettuce. This was recognized as *Bacterium viridilividum*, an organism known previously to produce a lettuce disease. This colony also proved to be infectious.

It appears that both of these organisms are present and active in soil in which there is abundant green manure or stable manure which has not been thoroughly decomposed. If conditions are such that the plant keeps up a steady growth and is not checked, these bacteria do not enter. When conditions are such that the plant is weakened or growth checked, an entrance is gained and disease follows.

The marginal disease of greenhouse lettuce reported from Kansas is also caused by a soil bacterium. The name "*Bacterium marginale*" is suggested. The margins of the inner whorl of leaves of immature plants are most frequently infected, but the entire leaf can be speckled or spotted by infection, which depends on defective greenhouse conditions. Subirrigation and proper ventilation will prevent this disease.

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XU

PLATE E

1.—*Bacterium viridilividum*, second organism isolated from Virginia lettuce: Appearance of the growth on potato at the end of 2 days.

2.—*Bacterium viridilividum*, original organism from Louisiana: Appearance of the growth on potato at the end of 2 days. Later, the upper part of the fluid becomes buff colored.

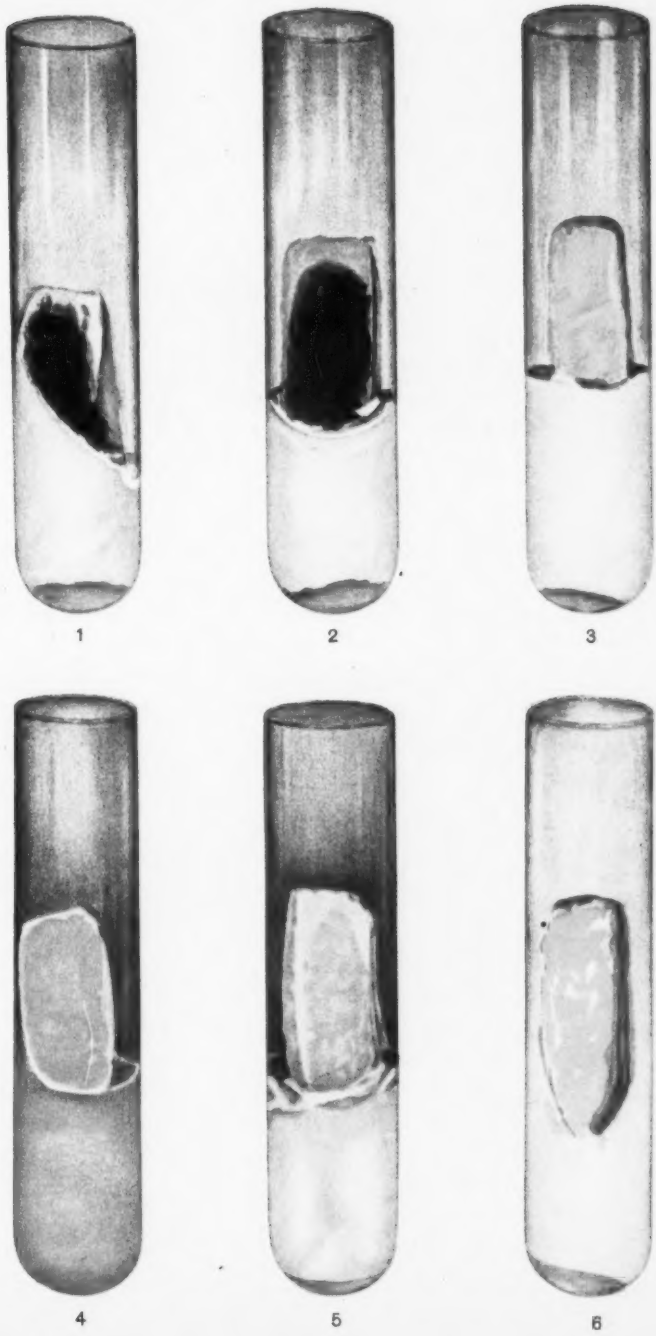
3.—*Bacterium vitians*, first organism isolated from Virginia lettuce: Appearance of the growth on potato at the end of 2 days.

4.—*Bacterium vitians*, isolated from South Carolina lettuce: Appearance of the growth on potato at the end of 3 days.

5.—*Bacterium marginale*, isolated from Kansas lettuce: Appearance of the growth on potato at the end of 2 days.

6.—*Bacterium marginale*, isolated from Kansas lettuce: Appearance of the growth on potato at the end of 13 days.

Painted by Mr. James F. Brewer.



1 2 3

4 5 6

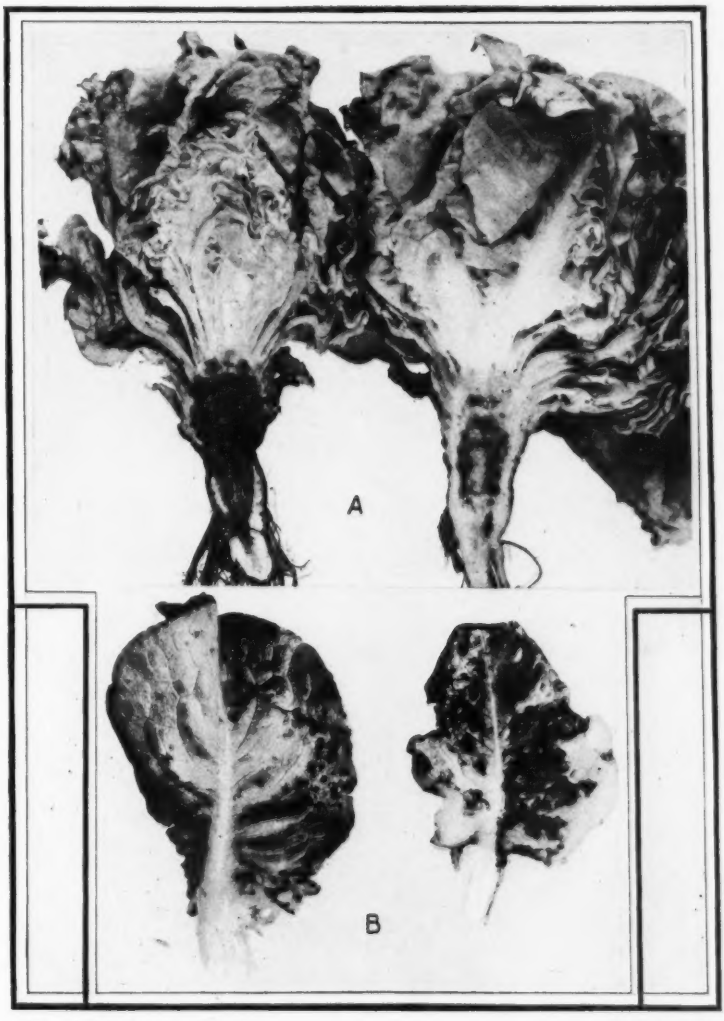
PLATE 29[1]

Bacterium vitians:

A.—Lettuce from Beaufort, S. C., showing stems blackened by the disease. Photographed by Dr. J. Rosenbaum.

B.—Lettuce leaves from South Carolina, showing spotted-leaf type of the disease. In many cases the stems were sound.

[1] Photographs and photomicrographs reproduced in Plates 29 to 41 were made by Mr. James F. Brewer, except as otherwise stated.


A
B

A
B

PLATE 30

Bacterium vitians:

A.—A field of 3½ acres of diseased lettuce at Beaufort, S. C. By actual count there are two good plants in a hundred. Photographed by Dr. J. Rosenbaum.

B.—A field of healthy lettuce at Beaufort, S. C. This field was planted with seed from the same lot as that sown in the field shown in A, but the land received different treatment previous to planting and afterward.

PLATE 31

Two badly diseased leaves of Virginia lettuce, from which both *Bacterium viridilividum* and the South Carolina yellow organism *Bact. vitians* were isolated.

A
B

PLATE 32

Bacterium viridilividum, the cause of the Louisiana lettuce disease:

A.—Three leaves of lettuce inoculated by needle pricks on February 15, 1915. Photographed on February 17, 1915. The tissues are blackened, and decay is progressing rapidly.

B.—Two pots of lettuce inoculated by spraying on February 19, 1915. Photographed on March 9, 1915.

PLATE 33

Bacterium marginale, the cause of the Kansas lettuce disease:

A.—A head of diseased lettuce from Manhattan, Kans. Photographed by Mr. L. E. Melchers.

B.—Single leaves of Manhattan lettuce, showing the effect of the marginal disease. Photographed by Mr. L. E. Melchers.

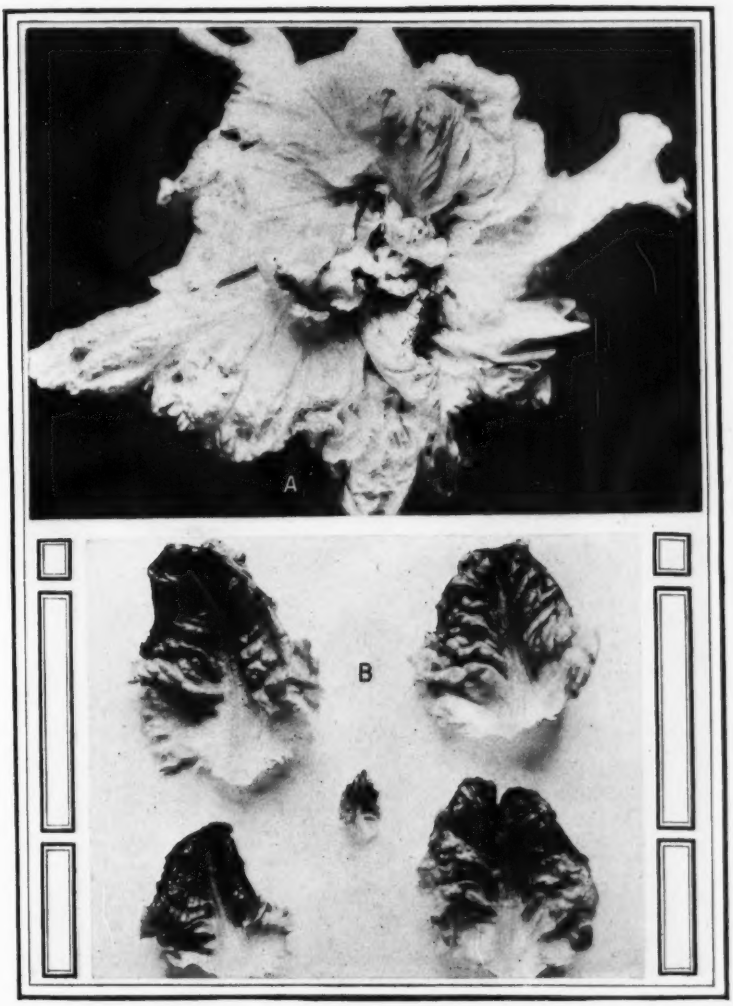

A
B

PLATE 34

Bacterium marginale:

A diseased leaf of lettuce received from Hutchinson, Kans. The organism producing this disease and that causing the marginal lettuce disease at Manhattan, Kans., are identical.

PLATE 35

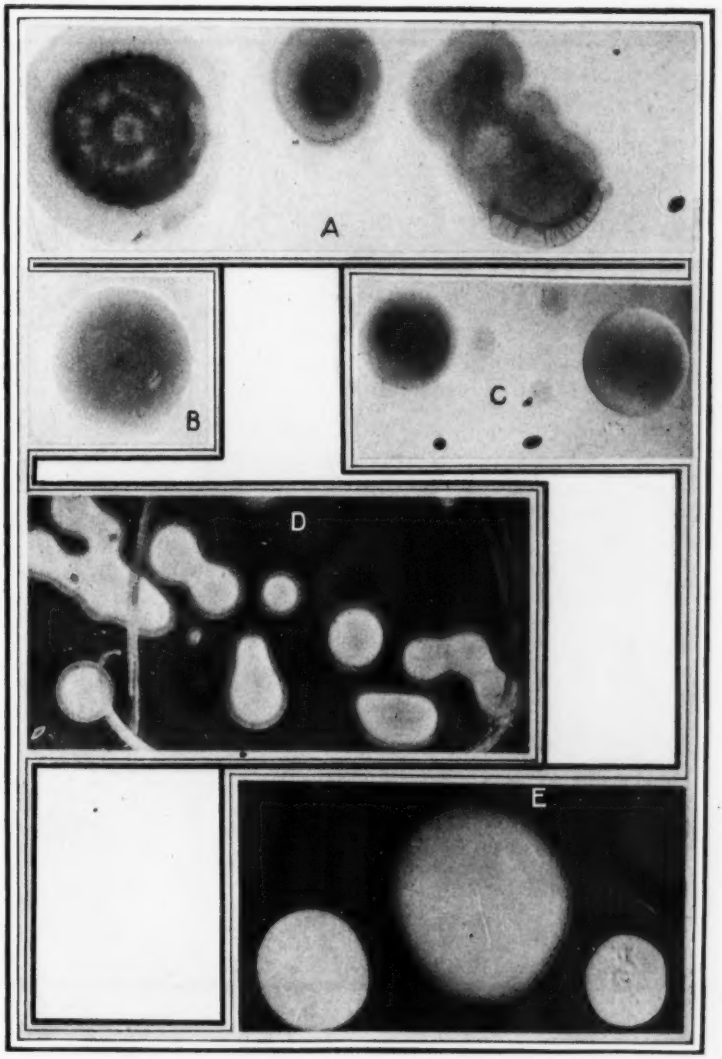
A.—Louisiana lettuce disease: Surface colonies on agar-poured plates of *Bacterium viridilividum*, showing the mottled type of colonies, and also one buried colony. Photographed at the end of six days. ×9.

B.—*Bact. viridilividum:* Nonmottled type three days after pouring. Both types of colonies are infectious. ×9.

C.—*Bact. vitians*, cause of the South Carolina lettuce disease: Agar-poured plate showing surface, buried, and bottom colonies. Photographed at end of the third day. ×9.

D.—*Bact. viridilividum*, cause of a Virginia lettuce disease: Mottled colonies on agar-poured plates. Photographed two days after pouring. The streak is a pencil mark on the outside of the dish. ×5.

E.—*Bact. marginale*, cause of the Kansas lettuce disease: Colonies on surface of agar-poured plates. Photographed three days after pouring. ×10.


A
B
C
D
E

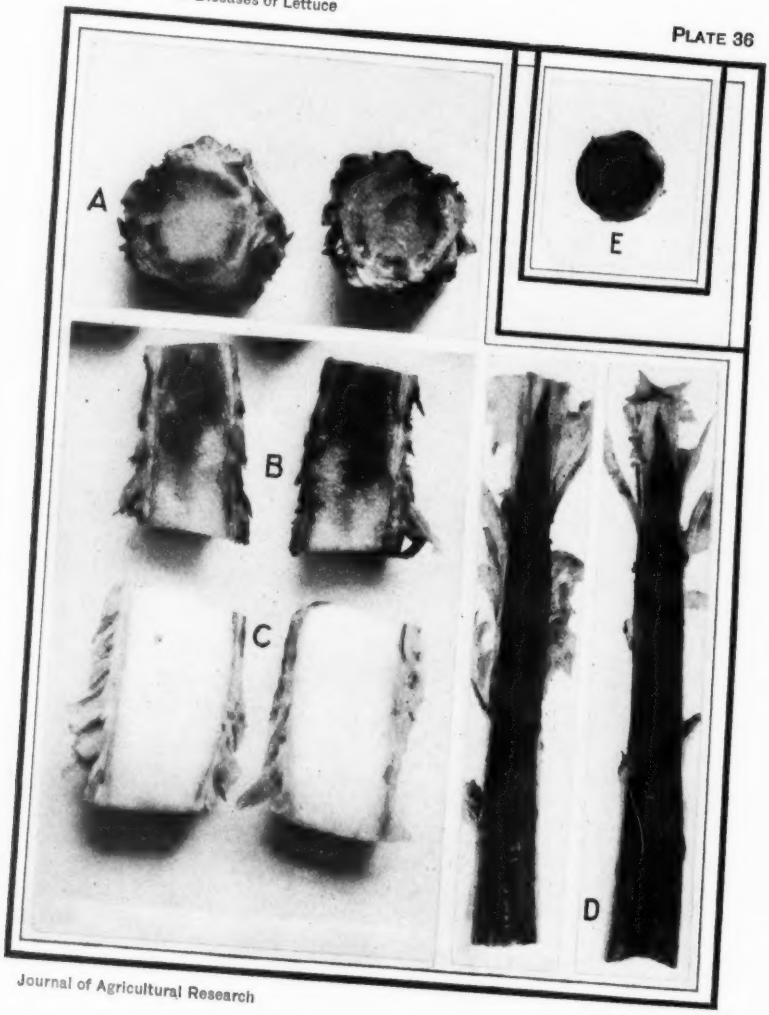

A
E
B
C
D

PLATE 36

Bacterium vitians the cause of the South Carolina lettuce disease:

A.—Cross-sections of a lettuce stem at two levels 35 days after inoculation with the South Carolina yellow organism. The tissues are browned.

B.—A longitudinal section of another plant inoculated at the same time as A.

C.—A longitudinal section of a healthy stem for comparison.

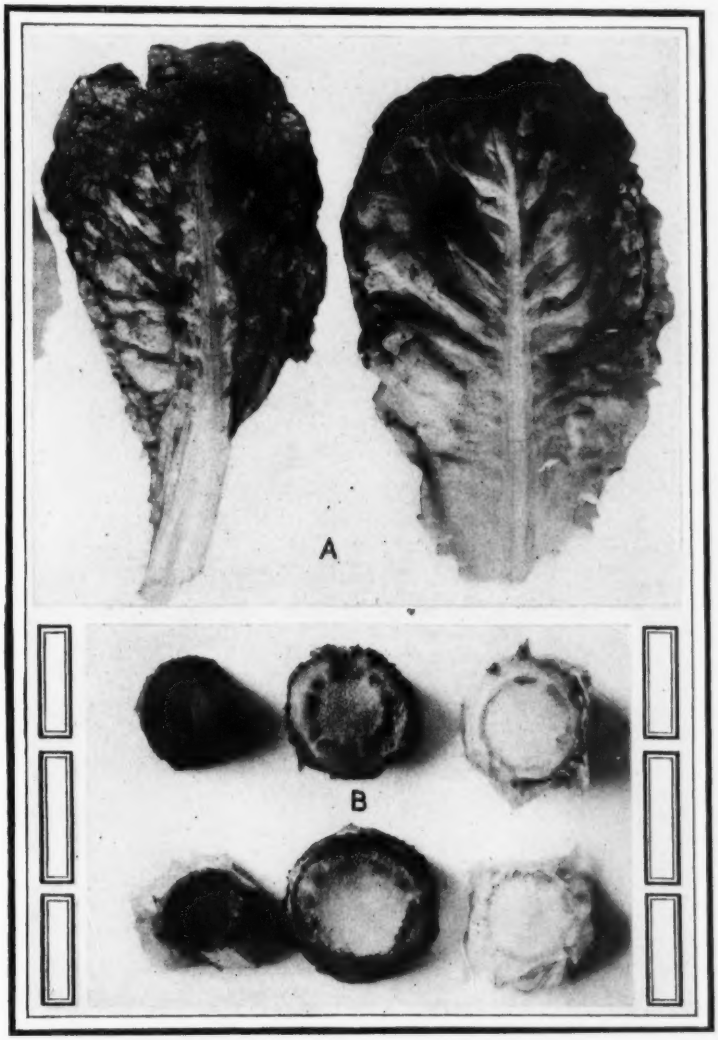
D.—Longitudinal sections at the crown of a lettuce plant one month after inoculation, showing browning of the tissues.

E.—A cross section at the crown of a lettuce plant one month after inoculation, showing browning of the tissues.

PLATE 37

A.—Two leaves of a lettuce plant inoculated by spraying with *Bacterium viridilividum* isolated from Virginia lettuce. Photographed 37 days after inoculation.

B.—Cross sections of stems of lettuce plants inoculated with the Virginia yellow organism (*Bact. vitians*), which is the same as the South Carolina lettuce organism. At the right sections of two healthy stems are included.


A
B

A
B

PLATE 38

Bacterium vitians:

A.—A lettuce plant inoculated by spraying with the Virginia yellow organism, which is the same as the South Carolina yellow organism. Photographed one month after inoculation.

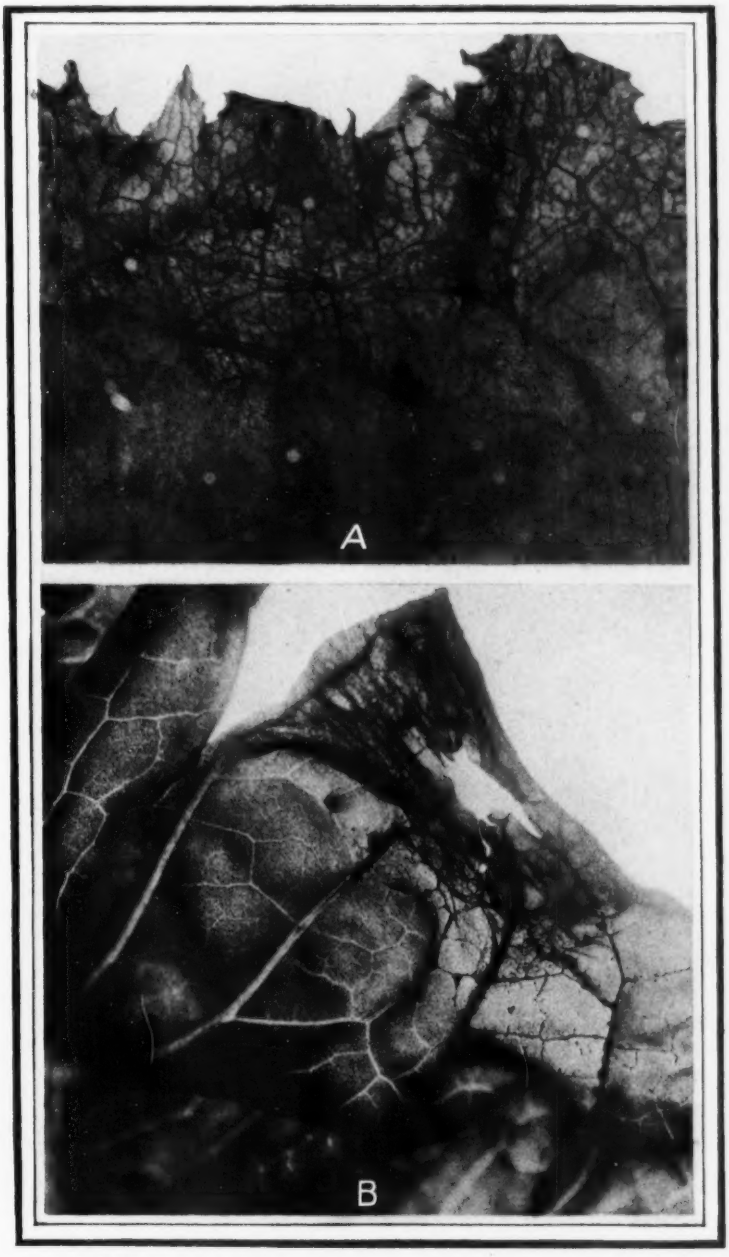
B.—Part of a healthy plant for comparison.

PLATE 39

Bacterium marginale:

A.—Part of a leaf from one of the original plants as received, showing the brown veins in the infected and shriveled margins. ×9 (about).

B.—Part of a lettuce leaf, showing the shriveling and the marginal brown venation produced by spraying with *Bact. marginale* on March 2, 1917. Photographed March 7, 1917. ×9 (about).


A
B

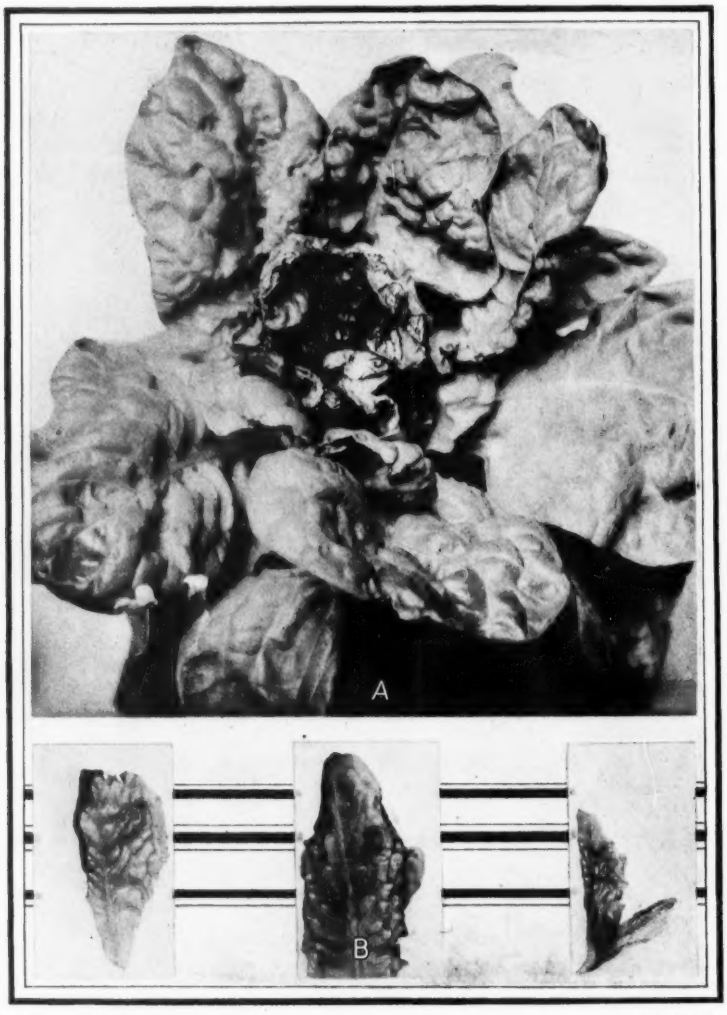

A
B

PLATE 40

Bacterium marginale:

A.—A head of lettuce showing the marginal infection on tender leaves in center. Inoculated by spraying on March 2, 1917. Photographed on March 16, 1917.

B.—Four lettuce leaves inoculated by spraying February 21, 1917. Photographed on February 23, 1917. Many of the infections are tiny spots not visible in the illustration—for example, on the leaf next to the smallest leaf at the right there are 75 such spots.

PLATE 41

A.—*Bacterium vitians:* Cross-section of stem showing bacteria in place. The organism has been stained with carbol fuchsin. ×1,000.

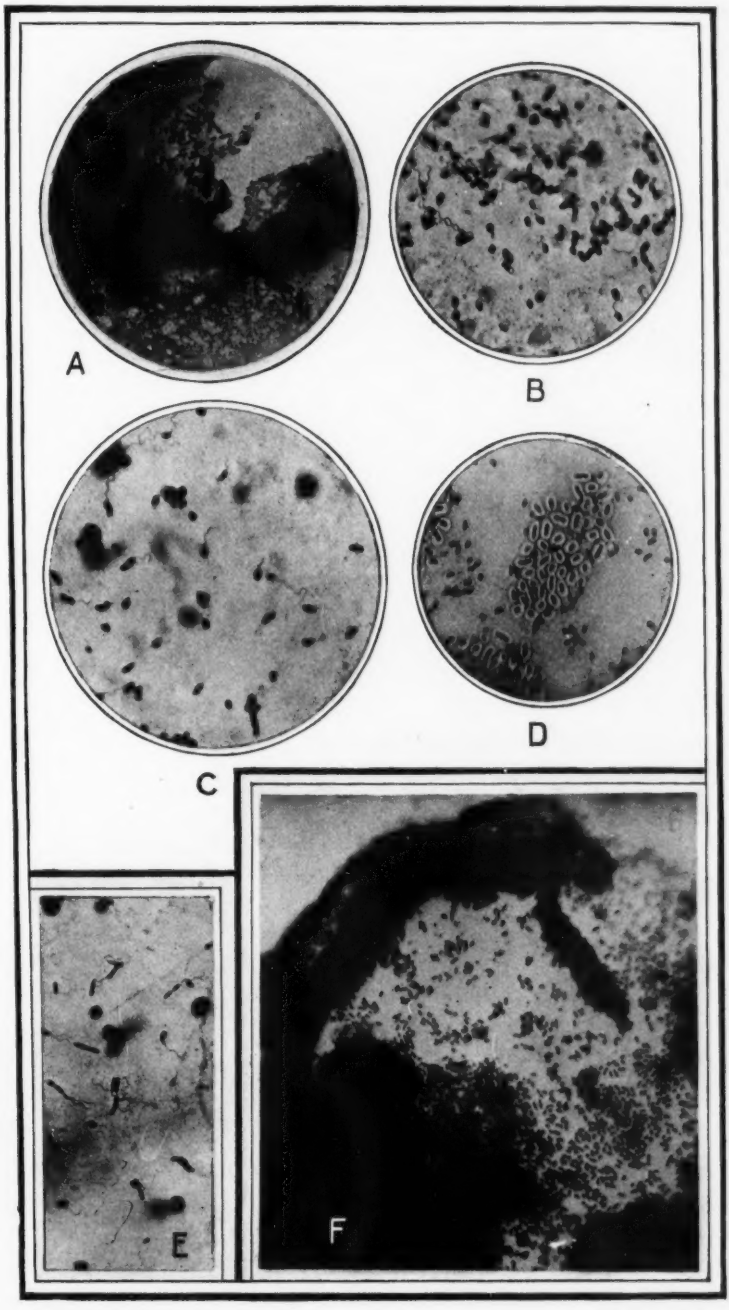
B.—*Bact. vitians:* Polar flagella stained with Casares-Gil's flagella stain; from a young agar culture. ×800 (about).

C.—*Bact. vitians* (Virginia): Polar flagella stained with Casares-Gil's flagella stain. Eighteen rods in this field bear flagella. ×800 (about).

D.—*Bact. marginale:* Grown on agar for two days and then stained with Ribbert's capsule stain. Photomicrographed by Dr. Erwin F. Smith. ×800 (about).

E.—*Bact. marginale:* Flagella stained with Casares-Gil's flagella stain. ×800 (about).

F.—*Bact. marginale:* Cross-section of a diseased, shriveled leaf showing bacteria in the tissues. Stained with carbol fuchsin. Photomicrographed by Dr. Erwin F. Smith. ×800 (about).


A
B
C
D
E
F